...25 NUMBER ...

...NT PHYSIOLOGY

JULY, 1950

CONTENTS

...ctions on the Absorption and Translocation of Inorganic ...ing Radioactive Isotopes with Plants. T. C. BROYER, ...y of California, Berkeley, California 367

... of Soil Temperature on Guayule Plants. H. M. BRYANT, ...tural Rubber Research Station, Salinas, California 377

...fect in Plant Growth-Regulators. CORWIN HANSCH and ...rn, Pomona College, Claremont, California, and ... ...y of Iowa, Iowa City, Iowa 389

... Nitrogen and Potassium Levels in Tung Seedlings on ...pparent Photosynthesis, and Carbohydrate Composition. ...TALOR, S. G. GILBERT, and M. DROSDOFF, Bureau of Plant ..., U. S. Department of Agriculture, Gainesville, Florida 394

...orption by Plants from an Atmosphere of High Humidity. ...BAUMAIR, W. T. MCGEORGE and J. F. BREAZEALE, Uni... of Arizona, Tucson, Arizona 413

... Correlations Between Growth Enlargement and the Solute ...ter Relations of Plants, with Special Emphasis on the Rela... Turgor Pressure to Distention of Cells. T. C. BROYER, ...ity of California, Berkeley, California 433

... American Elm (Ulmus Americana) Injured by Natural Gas ... CRISTAFORE, University of Michigan, Ann Arbor, Michigan ... 453

(Continued on Cover Page Four)

PUBLISHED QUARTERLY

BY

THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS

AT E. QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.

BUSINESS PRESS, INC.

Entered as second-class matter March 25, 1926, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

Accepted for mailing at the special rate of postage provided for in paragraph 4, section 538. Authorized April 9, 1926.

UNIVERSITY OF MICHIGAN LIBRARIES

PLANT PHYSIOLOGY

A QUARTERLY JOURNAL
DEVOTED TO ALL PHASES OF PLANT PHYSIOLOGY

Established 1926

EDITORIAL BOARD

WALTER F. LOEHWING, *Editor-in-chief*, 1954
State University of Iowa

C. S. PEARSON, 1955
Carnegie Institution
Stanford, California

FELIX G. GUSTAFSON, 1952
University of Michigan

H. B. VICKERY, 1951
Connecticut Agricultural
Experiment Station

F. P. CULLINAN, 1955
U. S. Department of Agriculture

PAUL J. KRAMER, 1953
Duke University

EZRA J. KRAUS, 1952
The University of Chicago

WALTER E. LOOMIS, 1951
Iowa State College

The pages of this journal are open to papers of merit dealing with any phase of Plant Physiology. Membership in the Society is not required of contributors, but non-members are required to pay a fee of $20.00 for each paper printed.

Manuscripts must be typewritten, and must have the approval of the Editorial Board. Papers must conform to the limitations established by the Society at the St. Louis meeting, 1926.

Proofs should be returned with necessary corrections, and reprints ordered when the proof is returned. Reprints can be furnished with or without covers at cost of production.

Correspondence with reference to editorial matters should be addressed to the Editor, Dr. Walter F. Loehwing, Department of Botany, State University of Iowa, Iowa City, Iowa.

Subscription price, $10.00 a year. Single copies, $3.00 post free. Members of the American Society of Plant Physiologists receive the journal in connection with dues paid to the Society.

Missing numbers will be supplied if lost in the mail, provided that written notice is received within one month of the date of issue; otherwise they will be furnished at the single copy rate, as long as they are available.

Correspondence concerning membership and its privileges, dues, change-of-address, etc., should be addressed to the Executive Secretary-Treasurer, Dr. J. Fisher Stanfield, Miami University, Oxford, Ohio.

Business correspondence regarding subscriptions, back numbers, changes-of-address of subscribers, etc., may be addressed to Plant Physiology, N. Queen St. and McGovern Ave., Lancaster, Pa., or to the Executive Secretary-Treasurer at the address above.

All matters pertaining to nominations, elections, programs, and meetings of the society should be addressed to the Secretary, Dr. T. C. Broyer, University of California, Berkeley, California.

PLANT PHYSIOLOGY

VOLUME 25 JULY 1950 NUMBER 3

FURTHER OBSERVATIONS ON THE ABSORPTION AND TRANSLOCATION OF INORGANIC SOLUTES USING RADIOACTIVE ISOTOPES WITH PLANTS

T. C. Broyer

(WITH THREE FIGURES)

Received May 1, 1950

Introduction

In earlier studies on the absorption of inorganic solutes by plants (**9**), the solute status of the system at the time of experimentation was shown to be of importance. Thus high-salt abscised roots were markedly restricted in further accumulation while with low-salt systems, rapid accumulation was subsequently evident. Where previously absorbed salt could move from the roots of high-salt plants to the shoots thereof, further inorganic solute could be readily absorbed from a nutrient medium. Thus it appeared that depletion of the previously accumulated salt in roots through translocation to the shoot allowed the continuance of the absorption process.

It has been shown also (**5**), with low-salt intact plants, that over short intervals of time the larger amount of salt absorbed was retained within the root system; presumably both in protoplasm and in vacuoles thereof, but particularly in the latter (cf. Exp. 2, here). Concurrent translocation to the shoot was limited, but took place more rapidly with time, following at least partial "saturation" of the roots relative to salt. A similar inference may be drawn from a study of decapitated plants, where the composition of exudates from the stumps indicates the movement of solute from root to shoot (**8**). Here, it was shown that salt movement to the upper portions of the plant was proportionally more rapid with previously treated (higher-salt) root systems. Again, for a period of several hours, a large portion of the absorbed salt, but decreasing with time, was retained within low-salt roots. With prolonged treatment, the exudates from both sets approached each other in solute composition indicating that after at least partial approach toward a maximum salt level within the cells of the root, a larger proportion of the inorganic solute currently absorbed, moved inward to the xylem and thence upward in the exudation stream.

UNIVERSITY OF MICHIGAN LIBRARIES

Other exudation studies (**3**) on the transport of solutes, followed by water, to the xylem of roots suggest that unless solute is rapidly replenished from an external source, the flow of solution as bleeding or guttation under favorable conditions from shoots, soon becomes negligible. Such a situation would arise particularly where a large proportion of the solute has moved, during previous treatment, into vacuoles of the cells intervening between the root surface and the xylem elements. Salt so resident appears to be subject to retention, that is, it does not readily migrate outward therefrom across cytoplasm to the bathing medium of cells. This may be the result of an impermeability of limiting surfaces (or the cytoplasm as a whole) to salt species *per se*, or if capable of ready outward diffusion, due to rapid concurrent reaccumulation thereof by actively metabolizing cells (**2, 4**). It should be recognized, however, that protracted migration may occur from vacuoles of roots. Thus a marked depletion of previously absorbed salt of roots through movement to the shoot may occur where further solute is withheld from the external supply (**11**).

It has been further suggested, on theoretical grounds (**1, 7**) and from inferences drawn from experimental data (**12**), that solute can move from cell to cell more readily by passage along continuous strands of protoplasm, where such a symplast exists. Thus, though salt in vacuoles is not readily translocated across roots, a ready overall pathway is afforded by the interconnecting protoplasm.

It was of interest to confirm, with the use of radioactive isotopes, the effect of the previous salt status of roots on the nature of subsequent accumulation of inorganic solute by abscised roots. Further, with the use of radioisotopes, a way seemed possible for testing the postulate that salt moves along a symplast pathway (at least in part) from cells near the surface of roots to the xylem. These studies are here reported.

Cultural treatment of plants

Barley plants were germinated, transplanted and allowed to grow in a glasshouse by a standardized technique described earlier (**9**). The inorganic solute supply was such that at the end of approximately three weeks, the plants were vigorous, yet in a low-salt, high-sugar condition. No forced aeration was applied during growth, to conserve carbohydrate within the plant systems. Sets of plants (168 plants per set) were subjected to experimental treatment as indicated below.

Experimental results and discussion

Experiment I.—Barley plants were separated into two lots. One group of plants was pretreated by exposing the roots to a KBr solution (25 milliequivalents per set) for a period of 16 hours. The second group was placed into water during the comparable interval of time. The roots of each group (approximately 100 gm. fresh weight per set) were then abscised and immersed in 3 liters of 0.00025 normal KBr*. The cultures

were aerated continuously during the experimental period. One pair of each group was subjected to a temperature of 0.5° C. a second to 20° C. Samples of the culture solutions were taken at successive intervals of time and measurements of radioactivity made thereon. Results of these measurements are recorded in the graph of figure 1. The curves show the relative decreases in radioactivity of the culture media (*i.e.*, the absorption by the roots) with time.

It may be seen from the graph, that more rapid absorption occurred at the higher temperatures both with the pretreated (plants with higher initial KBr concentrations) and the control roots. At the higher temperatures, nearly all of the radioactive bromide moved into the roots over a

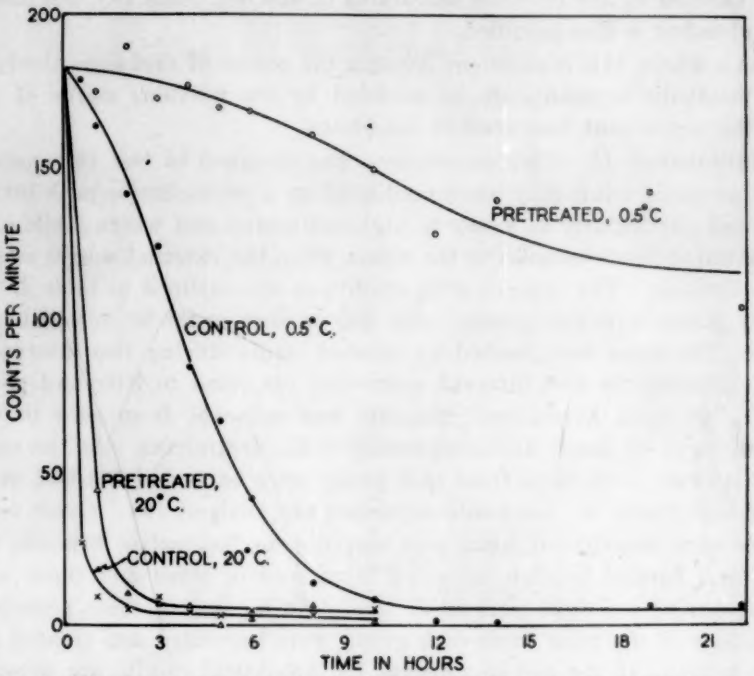

FIG. 1. Effects of pretreatment with inorganic solute and concurrent temperatures on the absorption of bromide by abscised barley roots.

short interval of time. No doubt this influx is related in large measure to metabolic accumulation, exchange adsorption playing a minor role. Regardless of the distribution of the absorbed solute in space within the roots, a very high accumulation ratio is evident.

The pretreated low temperature set shows definite absorption of radio bromide. This would seem to be most logically attributed to an exchange adsorption, particularly isotopic exchange between the protoplasm, together with cell surfaces, and the external medium. The control roots at the lower temperature display a greater accumulation relative to the pre-

UNIVERSITY OF MICHIGAN LIBRARIES

treated group and less than either set at higher temperatures. This pronounced influx at lower temperature is probably related to a large extent to metabolic accumulation, exchange adsorption being of the order indicated for the low temperature, pretreated roots, or even less, since the possibility of isotopic exchange is excluded. These data are in accord with earlier evidence that metabolic accumulation can proceed, though more slowly, even at low temperatures (**6**), as well as with those indicating ready exchange under similar conditions (**6, 10**).

Another feature is evident from these curves. The movement of radioactive bromide is less rapid into the pretreated roots which have a higher initial salt level. Thus it becomes clear, that not only is the accumulation level affected by the previous salt status of abscised roots (**9**), but also the rate of influx is thus modified.

As a whole, this experiment delimits the course of exchange absorption and metabolic accumulation as modified by the previous status of roots and the concurrent temperature conditions.

EXPERIMENT II.—This experiment was designed to test the postulate that inorganic solute may move readily along a protoplasmic path between cells and particularly as a way in high-salt tissues and where labile salt is translocated from vacuoles to the xylem when the external source may become limiting. The experimental conditions are outlined in table I. The barley plants were decapitated such that exudate could be collected therefrom. The roots were bathed by aerated media during two intervals of time. During the first interval, part were subjected to KBr and part to $CaSO_4$, of equal normality. Exudate was collected from zero to three and three to six hours and composited within treatments. At the end of these periods, some roots from each group were harvested, washed, centrifuged and frozen for composite expressed sap analysis (**9**). Other sets of plants were transferred, after root washing, to radioactive bromide solutions and further exudate collected from zero to three and three to six hours, during a second interval of time. At the end of these periods the remainder of the roots from each group were harvested and treated similarly to those at the end of interval 1. Analytical results are presented in table I and particularly pertinent data are graphically shown in figures 2 and 3.

Certain general features may be pointed out before detailed discussion of the graphs. It has been pointed out in the introduction, from earlier study with exudates (**3**), that unless concurrent accumulation of labile solutes takes place, the rate of exudation decreases with time. This situation may be observed here as well, where $CaSO_4$ and KBr treated plants are compared. (cf. column 4, and 7 and 10). $CaSO_4$ is slowly accumulated while KBr rapidly migrates into root cells, and into the xylem where the solute concentration relative to the external medium is effective in causing exudation to tend to occur. With some species of plants, little or no exu-

TABLE I

Sets	Experimental conditions			Volumes	Br*		Specif. cond.	Bromide		K
	Interval 1	Interval 2	Sample	Sap or exudate per set ml. cor.[1]	C/m/ ml.	tc/m²	Mhos $\times 10^4$	M.e./l	T m.e.	M.e./l
(Columns)	1	2	3	4	5	6	7	8	9	10
1	Control roots		Roots	79.	.....	.....	48.3	.....	.....	35.1
2	KBr 3 hrs.		Roots	68.	.....	.....	73.0	12.1	0.82	63.8
			Exudate	10.0	.....	.....	24.8	10.1	0.10	11.8
3	KBr 6 hrs.		Roots	72.	.....	.....	71.4	16.2	1.17	65.5
			Exudate	12.7	.....	.....	29.5	17.7	0.23	17.3
4	KBr 6 hrs.	KBr* 3 hrs.	Roots	78.	300.	23400.	79.3	22.2	1.73	72.8
			Exudate	13.9	650.	9025.	35.7	27.0	0.38	35.6
5	KBr 6 hrs.	KBr* 6 hrs.	Roots	75.	510.	38200.	91.7	31.6	2.37	82.9
			Exudate	13.8	896.	12350.	44.6	30.5	0.42	.....
6	$CaSO_4$ 3 hrs.		Roots	75.	.....	.....	51.0	.....	.....	36.6
			Exudate	4.4	.....	.....	19.7	.....	.....	5.9
7	$CaSO_4$ 6 hrs.		Roots	74.	.....	.....	49.7	.....	.....	37.2
			Exudate	4.8	.....	.....	13.6	.....	.....	3.7
8	$CaSO_4$ 6 hrs.	KBr* 3 hrs.	Roots	70.	638.	44700.	73.0	19.4	1.36	63.5
			Exudate	12.3	632.	7770.	27.5	17.5	0.22	17.0
9–10	$CaSO_4$ 6 hrs.	KBr* 6 hrs.	Roots	81.	860.	69600.	81.2	25.2	2.04	66.7
			Exudate	14.5	858.	12420.	38.9	25.4	0.37	23.7
	KBr solution							4.3	12.8	4.5
		KBr* solution			185.	555000.		4.8	14.5	5.83

[1] Per 167 plants, each set.
[2] Total counts per minute Br* per set (167 plants) per 3 hr. period.
All cultures approximately 0.005N; 3 liters per pan of 167 plants each.

UNIVERSITY OF MICHIGAN LIBRARIES

date is obtained, even over short intervals of time, from low-salt plants under otherwise favorable conditions (3). Where the $CaSO_4$ medium is subsequently replaced by KBr*, the exudate concentration and rate of flow are concurrently increased. As shown earlier, marked accumulations of solutes arise in the xylem relative to certain dilute bathing media (cf. columns 7, 8 and 10, here). As noted elsewhere (3), there is a high indi-

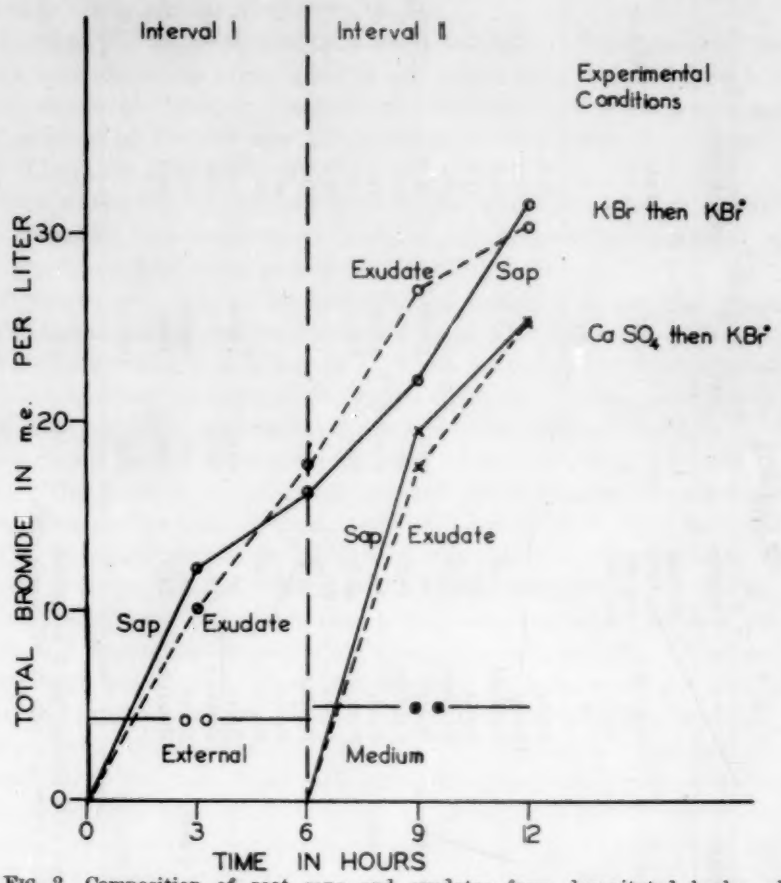

FIG. 2. Composition of root saps and exudates from decapitated barley plants with time; total (non-radioactive plus radioactive) bromide concentration in milliequivalents per liter of solution. For experimental conditions, see table I.

vidual variability in exudation rate (less in composition of sap) among a lot of plants of the same species, cultured similarly. In the current study, the numbers of plants are sufficiently great to make the noted differences of significance.

In figure 2, the total bromide concentrations in exudates and composite expressed saps of the roots are plotted against time. A large portion of the cells of the root are highly vacuolate and it is assumed that the com-

posite sap concentrations nearly equal those of the vacuoles in magnitude which seems reasonable if at any particular time there is a tendency toward constrained dynamic equilibrium for solute and water among the external and internal media and the interposed protoplasm. There is quite close agreement at each of the time periods between the total bromide concentrations in the exudate and in the root sap. The measured values are plotted at the end of the particular time period. The exudates

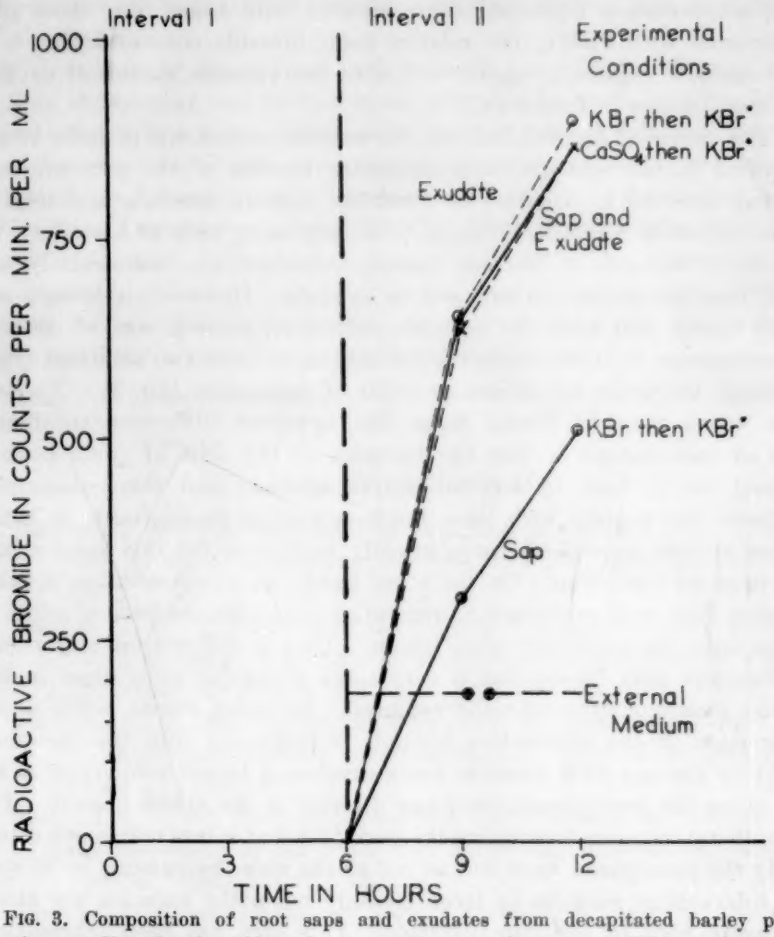

FIG. 3. Composition of root saps and exudates from decapitated barley plants with time; radioactive bromide only, in counts per minute per milliliter of solution. For experimental conditions, see table I.

were collected throughout the time period, therefore, the composition represents an average for the time involved, while the sap values represent those at the end of the corresponding period. For this reason, one might have expected the sap values to possibly exceed those of the exudates as plotted, however this is not significantly the case. It would seem that these close correspondences in magnitude would obtain if the inorganic

UNIVERSITY OF MICHIGAN LIBRARIES

solute (accompanied by water, osmotically) on influx moves rather proportionally into lumena of both the vacuoles of low-salt root cells and the xylem. Although the relative distances to these two types of lumena may be considerably different, the permeabilities of the effectively interposed protoplasm and energy intensity gradients may be of similar orders of magnitude for low-salt roots. At all events, the data indicate movement of total bromide into the vacuolate cells and into the stelar vessels such that accumulation levels are quite parallel with time. For those plants pretreated with $CaSO_4$, the relative total bromide concentrations in sap and exudate (figure 2) agree well with the bromide measured by radioactivity (shown in figure 3).

The curves of figure 3 indicate the relative movements of radio-bromide supplied to two systems—now dissimilar because of the preconditioning during interval 1. One set is relatively high in bromide and total salt while the other is relatively low in total salt, being without bromide. With supply of bromide to low-salt tissues, as before, the radioactivity levels with time are similar in sap and in exudate. However, it is very strikingly shown that with the bromide pretreated roots a marked difference in radioactive bromide concentration obtains in these two solutions (fig. 3) although the totals are of similar order of magnitude (fig. 2). The inference which may be drawn from this apparent difference in distribution of radioisotope is that the vacuoles of the cells of these roots are already fairly high in non-radioactive bromide and this concentration increases less rapidly with time, for it is tending to approach, in being a closed system, a level characteristically maximum for this species under the imposed conditions. On the other hand, the xylem solution is subject to mass flow, with continued movement, to replenish the loss, of solute and water into the relatively open lumen. Thus a differential migration of radioisotope may be envisioned across and along the protoplasm in favor of the plasma continuum—the symplast. In other words, while a small proportion of the radioactive bromide is migrating into the vacuoles to build up the sap total bromide concentration, a larger proportion is moving along the protoplasmic pathway directly to the xylem lumena. These data, therefore, seem to support the postulate that solute may move directly along the protoplasm from cell to cell to the stele, by-passing, so to speak, the intervening vacuoles in large measure when the vacuoles are already relatively high in salt concentration. This idea has been schematically represented elsewhere, in a diagram of two possible plant systems for solute movement (1, fig. 1). That such relatively independent movement can take place seems clear from the present results.

Summary

With the use of abscised barley roots immersed in radioactive bromide solutions, it has been possible to more closely delimit the modes of migration of electrolyte into tissues at low and high temperatures as well as into

systems of previously high- or low-salt content. This study suggests several points. First, there is a rapid exchange of isotopes between the external medium and roots; second, under favorable temperature conditions isotopic or ion exchange is relatively less important than migration by metabolic accumulation; third, that with high-salt roots at low temperature, migration may be restricted almost exclusively to adsorption exchange; and fourth, that metabolic accumulation may be pronounced even with roots at low temperature, provided they are currently in a relatively low-salt condition.

With the use of decapitated barley plants and isotopes of bromide it has been shown that two pathways are afforded for the migration of inorganic solutes into roots. First, with low salt plants, salt may migrate across the cytoplasm of cells into vacuoles as well as to more remote lumena, *e.g.*, the xylem. Second, where the roots are initially in a relatively high-salt condition, subsequent migration takes place differentially along continuous protoplasmic paths (the symplast) to the xylem, circumventing, in a measure, movement into or through intervening vacuoles. Where depletion of vacuolar solute occurs through translocation, the probable pathway is likewise along the protoplasmic continuum.

DIVISION OF PLANT NUTRITION
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BROYER, T. C. The movement of materials into plants. II. The nature of solute movement into plants. Bot. Rev. **13**: 125–167. 1947.
2. BROYER, T. C. The nature of the process of inorganic solute accumulation in roots. In Symposium on Mineral Nutrition of Plants. University of Wisconsin Press. Madison, Wisconsin. 1950.
3. BROYER, T. C. Studies on the movement of water into plants with emphasis on a possible metabolic factor. Amer. Jour. Bot. **37**:— 1950.
4. BROYER, T. C. A generalized comprehensive scheme for discussion of the movement of materials into living cells and tissues. J. Gen. Physiol. 1950.
5. BROYER, T. C. and HOAGLAND, D. R. Metabolic activities of roots and their bearing on the relation of upward movement of salts and water in plants. Amer. Jour. Bot. **30**: 261–273. 1943.
6. BROYER, T. C. and OVERSTREET, R. Cation exchange in plant roots in relation to metabolic factors. Amer. Jour. Bot. **27**: 425–430. 1940.
7. CRAFTS, A. S. and BROYER, T. C. Migration of salts and water into xylem of the roots of higher plants. Amer. Jour. Bot. **25**: 529–535. 1938.

UNIVERSITY OF MICHIGAN LIBRARIES

8. HOAGLAND, D. R. Salt accumulation by plant cells, with special reference to metabolism and experiments on barley roots. Cold Spring Harbor Symp. on Quant. Biology. **8**: 181–194. 1940.
9. HOAGLAND, D. R. and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
10. JACOBSON, L. and OVERSTREET, R. A study of the mechanism of ion absorption by plant roots using radioactive elements. Amer. Jour. Bot. **34**: 415–420. 1947.
11. STEWART, F. C., PREVOT, P. and HARRISON, J. A. Absorption and accumulation of rubidium bromide by barley plants. Localization in the root of cation accumulation and of transfer to the shoot. Plant Physiol. **17**: 411–421. 1942.
12. WIERSUM, L. K. Transfer of solutes across the young root. Recueil des Travaux botaniques neerlandais. **41**: 1–79. 1946–7.

THE EFFECT OF SOIL TEMPERATURE ON GUAYULE PLANTS

H. M. BENEDICT[1]

(WITH SIX FIGURES)

Received October 31, 1949

Introduction

The work of several investigators, including BONNER (**2**), WHITEHEAD and MITCHELL (**18**), has shown that low air temperatures greatly reduce the growth rate of guayule plants and at the same time bring about an increase in their rubber percentage and, conversely, that high air temperatures increase the growth and decrease the rubber percentage. However, aside from two brief unpublished reports (**4, 6**) little information is available as to the effect of soil temperatures on the growth, carbohydrate and rubber production of guayule. This paper presents results obtained where guayule plants were grown at the same air temperature but at different soil temperature levels.

Methods

During the experiments, all the plants were grown in soil in 5-gallon metal cans. These cans were submerged in water in the controlled temperature tanks described by CAMPBELL and PRESLEY (**7**) and located in the greenhouse. Twelve tanks, each holding eight cans, were available, four of these 12 were refrigerated and eight heated. For the most part two of the tanks, holding a total of 16 cans, were maintained at the same temperatures. This allowed a total of six different soil temperatures to be maintained during one experiment. When the plants were harvested, they were grouped into four replications of four plants each. The temperature of the soil in the cans was held within two degrees of that desired. The air temperature averaged about 76° F, with a minimum of 70° at night and a maximum of 90° during the day.

In the first experiment, 5-month-old seedlings were trimmed back to a single stem about four inches long and $\frac{3}{16}$ of an inch in diameter and transplanted on February 12, 1947 to the soil in the cans. This resulted in a fairly uniform set of plants at the start of the experiment. The plants were all grown for six weeks at a uniform soil and air temperature to give them a good start, then grown from April 1 to June 10 in soil temperatures of 40, 50, 65, 70, 80 and 90° F and the air temperatures of 76° F. On June 10, the plants were washed from the soil and their dry weight, moisture, carbohydrate, resin and rubber content determined.

[1] Present address: Isotopes Division, Atomic Energy Commission, Oak Ridge, Tennessee.

UNIVERSITY OF MICHIGAN LIBRARIES

In the second experiment, plants that had been seeded in greenhouse benches in February 1947, grown until July 1947, then hardened off by withholding water until the middle of November, were used. These plants were cut back to a single stalk about four inches long and $\frac{5}{16}$" in diameter and transplanted to the cans on November 15, 1947. The plants were grown at constant soil temperatures of 70° until January 2, 1948 when the differential soil temperatures were applied.

These consisted of 45, 55, 65, 75, 85 and 95° F and were imposed on the plants from January 2, 1948 until they were harvested on May 1, 1948. At the time of harvest, dry weights, moisture, carbohydrate, resin and rubber contents were determined.

The soils used in the two experiments were quite different. That of the first, classified as a type of Salinas fine sandy loam non-calcareous phase, was a dark soil that gave a very rapid initial but much reduced later growth. That of the second, classified as Greenfield coarse sandy loam, gave a steady and fairly rapid growth over a long period of time.

At the time of the harvest, the leaves were picked from the plants by hand, the stems and roots separated, and the various determinations made on all three plant parts. The dry weights and moisture contents were determined by drying the plants to a constant weight in a ventilated oven at 65° C. The water-soluble carbohydrates consisting of the free sugars, levulins, and inulin were determined by the method of TRAUB and SLATTERY (17), while total pentosans were estimated according to the method of MCRARY and SLATTERY (11). Resins and rubber hydrocarbon were determined according to the method of TRAUB (15). Analyses of variance were carried out on the data and the difference required for significance at the 5% level, calculated according to the methods described in HAYES and IMMER (9).

Data

In presenting the data the dry weights of the flowers, leaves, stems, and roots are given. The percentage content of the various materials in the leaves was determined but, with the exception of the total pentosans which averaged about 10%, the content of these materials was small and did not vary significantly with treatments. Therefore, for the sake of brevity, the results of the analyses on the leaves have been omitted.

The dry weight of the various parts of the plant that grow above ground, *i.e.*, flowers, leaves, and stems, increased with increased soil temperature up to 80 or 85° F in both experiments, then decreased with further increase in temperature. The maximum dry weight of the roots, however, was obtained at a soil temperature of 65° F in both experiments, although the dry weights at 70° and 75° did not differ significantly from those at 65° (fig. 1). The plants in experiment 1 did not make nearly the growth of those in experiment 2. This could be due to the longer duration of the growth period, but the smaller differences between the

maximum and minimum dry weights in experiment 1 suggest that some other factor, perhaps a soil deficiency, was limiting the growth in the more favorable temperatures in this experiment. Soon after the differential soil temperatures were applied to the plants of the first experiment they exhibited signs of nitrogen deficiency. The same amount of ammonium phosphate was then added to the plants in all the treatments and the nitrogen deficiency symptoms disappeared and did not recur. This might be considered as additional evidence that some factor related to the soil was retarding the growth of the plants in experiment 1, especially at the higher temperatures, as at the lower temperature the dry weights of the plants in the two experiments did not differ greatly from each other.

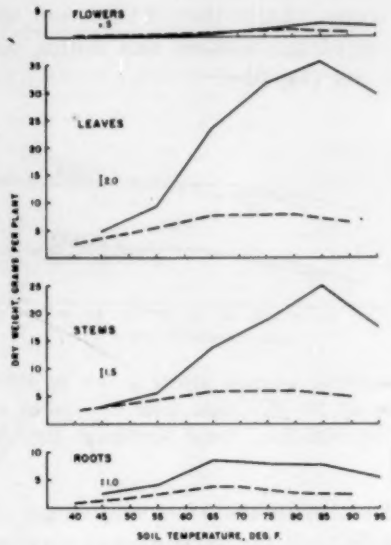

FIG. 1. Dry weight of guayule plants grown in different soil temperatures but at an air temperature of 75° F. Data from experiment 1 are shown by broken line; from experiment 2, by solid line. Least significant difference $P < .05$ represented by "I" bar.

In experiment 1, when the differential temperatures were first applied, the plants in the 40, and 50° tanks quickly wilted, in spite of the soil being very wet, and remained so for about four days. As a result of this observation, the moisture percentage of the plants in the various treatments was closely observed at the time of harvest. The results in figure 2 show that the moisture content of all parts of the plants was lowest at the lowest soil temperature, but reached a maximum at about 65° or 70° F. The moisture contents at the highest soil temperatures, except in the leaves, were usually significantly lower than at 65° but not nearly as low as those at 40° and 45°. Results seem to indicate that the guayule plants have difficulty in absorbing sufficient water at both low and high tem-

UNIVERSITY OF MICHIGAN LIBRARIES

peratures but that much greater difficulty is encountered at the low temperature.

Except for the free sugar content of the stems, which did not differ, the percentages of free sugars, levulins, and inulin in both experiments were highest at the lowest soil temperature, decreased rapidly as the soil temperature was increased to 65° F, but did not change significantly with further increases in soil temperature. The pentosan content, on the other hand, was lowest at the lowest soil temperatures, reached a maximum at 65° and 70° F, then showed no change as the soil temperature was further increased (table I). The percentages of the various carbohydrates were generally considerably higher in the stems than in the roots.

No very general statements can be made concerning the grams per plant of the carbohydrates, except that the low soil temperatures seem to favor the accumulations of the levulins and inulin, which are the important carbohydrate reserves (**14, 16**).

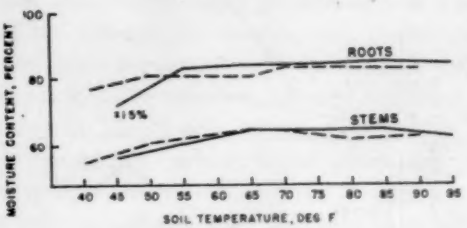

FIG. 2. Moisture content of guayule plants grown in different soil temperatures but at an air temperature of 75° F. Data from experiment 1 are shown by broken line; from experiment 2, by solid line. Least significant difference $P < .05$ represented by "I" bar.

The percentage of resins in the roots and stems of experiment 1 was lowest at the 65° and 70° soil temperature and increased as the temperature was raised or lowered. In experiment 2, the highest resin percentage was found at the lowest soil temperature and gradually decreased as the soil temperature increased (fig. 3). The actual amount of resins per plant was greatest in both experiments at soil temperatures of 80° to 85° in the stems and at 65° in the roots (fig. 4).

The rubber percentage in the stems and roots in the first experiment decreased as the soil temperature was increased from 40° to 65° F, then increased as the soil temperature was increased from 65° to 90°. In the second experiment, the rubber percentage continued to decrease as the soil temperature was increased from 45° to 95° (fig. 5). In spite of the different behavior of the rubber percentages in the two experiments, the maximum amount of rubber on a grams-per-plant basis was produced in next to the highest temperatures, *i.e.*, 80° and 85° F, with the exception of the roots of the plants in the second experiment when the maximum amount was produced at a soil temperature of 65°. However, even in

this instance, the amount produced in the stems at the soil temperature of 85° was so much greater than at 65° that the total amount produced by the entire plant was greatest at 85° F (fig. 6).

Discussion

In discussing the results, it seems necessary to stress the difference in growth responses of the plants in the two experiments. The dry weights

TABLE I

PERCENTAGE OF CARBOHYDRATES IN GUAYULE PLANTS GROWN IN VARIOUS SOIL TEMPERATURES

PLANT PART AND SOIL TEMPERATURE	CARBOHYDRATE CONTENT							
	FREE SUGARS		LEVULINS		INULIN		PENTOSANS	
	EXPT. 1	EXPT. 2	EXPT. 1	EXPT. 2	EXPT. 1	EXPT. 2	EXPT. 1	EXPT. 2
	%	%	%	%	%	%	%	%
Stems								
Degree F								
40	.38		8.67		3.50		16.63	
45		.61		8.47		2.20		17.36
50	.46		6.23		1.15		17.65	
55		.63		4.31		.34		19.99
65	.35	.52	2.34	1.50	.39	.24	18.45	20.31
70	.38		1.88		.36		18.22	
75		.64		1.41		.16		19.89
80	.35		2.70		.27		18.05	
85		.55		1.24		.13		19.66
90	.35		2.71		.61		18.36	
95		.68		1.88		.14		19.82
*L.S.D. $P < .05$	.28	.29	1.13	1.24	.41	.77	.42	.48
Roots								
Degree F								
40	.61		4.32		1.60		14.57	
45		.98		10.39		1.80		14.77
50	.59		3.55		.77		15.15	
55		.86		6.69		.83		14.85
65	.21	.25	.57	.79	.23	.05	14.18	15.18
70	.14		.33		.10		15.00	
75		.27		.62		.04		15.17
80	.31		.27		.42		16.70	
85		.36		.56		.03		15.85
90	.22		.62		.40		15.42	
95		.50		.94		.07		16.33
*L.S.D. $P < .05$	.28	.29	1.13	1.24	.41	.77	.42	.48

* Least significant difference.

of the various parts of the plants did not differ significantly from each other at the lowest soil temperatures but as the soil temperature was raised the difference between the plants in the two experiments greatly increased, those of experiment 2 having increasingly greater dry weight than those of experiment 1. As the soil temperature increased beyond 65° F, the above-ground parts of the plants of experiment 1 showed barely significant increases in dry weight, while those of experiment 2 showed

UNIVERSITY OF MICHIGAN LIBRARIES

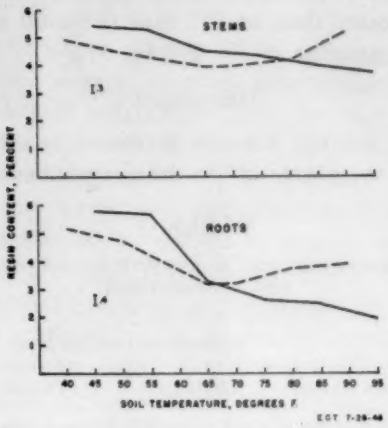

FIG. 3. Resin percentage of guayule grown in different soil temperatures but at an air temperature of 75° F. Data from experiment 1 are shown by broken line; from experiment 2, by solid line. Least significant difference $P < .05$ represented by "I" bar.

very great increases. These results indicate that at the lower temperatures the rate of growth in both experiments was limited primarily by the soil temperature, but at the higher temperatures that some additional fac-

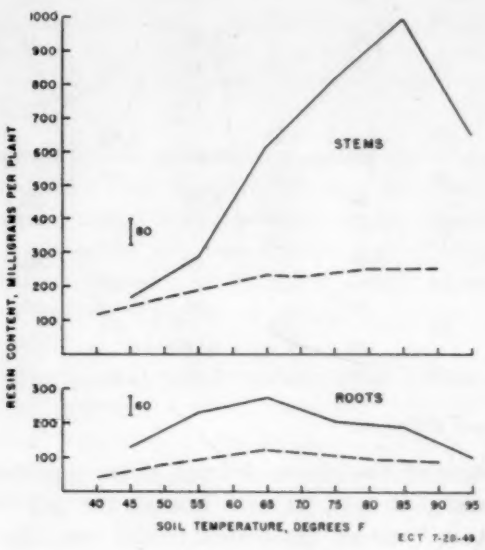

FIG. 4. Grams of resin in guayule plants grown in different soil temperatures but at an air temperature of 75° F. Data from experiment 1 are shown by broken line; from experiment 2, by solid line. Least significant difference $P < .05$ represented by "I" bar.

tor was suppressing the growth of the plants in experiment 1 and not in experiment 2. This factor must be kept in mind in considering the results obtained with the various constituents.

In spite of the differences in growth rates, the results of the two experiments in an air temperature of 75° F indicate that a soil temperature of 80 to 85° F is optimum for the growth of the above-ground parts (flowers, leaves, and stems) of guayule plants, and 65° F is the optimum soil temperature for root growth. ERICKSON and SMITH (8) obtained the greatest dry weight of new roots and top growth of transplants in nutrient solution after three weeks at 70° F and the greatest top growth in soil at 78.5° F. These results are in fairly close agreement with those obtained here. ERICKSON and SMITH (8) have published data on the soil temperature at three different depths and the air temperatures at Salinas from

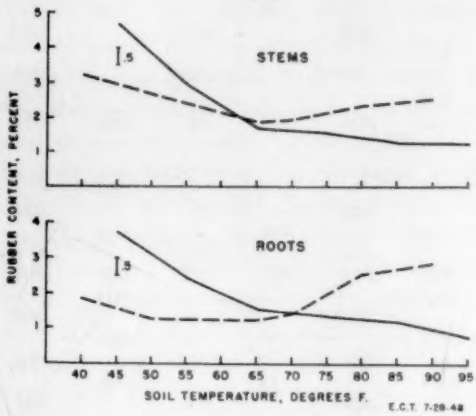

FIG. 5. Rubber percentage of guayule plants grown in different soil temperatures but at an air temperature of 75° F. Data from experiment 1 are shown by broken line; from experiment 2, by solid line. Least significant difference $P < .05$ represented by "I" bar.

September 1944 through March 1945. During this period, the soil temperature was below 65° F; assuming that this was not far from an average year, these low soil temperatures probably contributed to the slow growth made by the guayule plants during this season of the year at Salinas.

The action of the low temperatures in slowing down the growth is, of course, related to the direct effect of temperature on various chemical reactions. In addition, the wilting of the plants at the start of the experiment and the low moisture content of the plants suggest that, even though abundant moisture is present in the soil, the roots cannot take it in rapidly enough to meet the demands of the tops, especially when they are in a higher temperature than the roots.

The percentage content of the various constituents show two rather distinct trends. Regardless of the growth made by the plants, particu-

UNIVERSITY OF MICHIGAN LIBRARIES

TABLE II

GRAMS OF CARBOHYDRATE PER GUAYULE PLANT GROWN IN VARIOUS SOIL TEMPERATURES

PLANT PART AND SOIL TEMPERATURE	GRAMS PER PLANT OF DESIGNATED CARBOHYDRATE AND EXPERIMENT NUMBER							
	FREE SUGARS		LEVULINS		INULIN		PENTOSANS	
	EXPT. 1	EXPT. 2	EXPT. 1	EXPT. 2	EXPT. 1	EXPT. 2	EXPT. 1	EXPT. 2
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Stems								
Degree F								
40	.009		.208		.085		.399	
45		.018		.255		.066		.522
50	.017		.228		.042		.646	
55		.034		.236		.019		1.093
65	.021	.071	.139	.206	.023	.033	1.094	2.785
70	.022		.109		.021		1.057	
75		.120		.266		.030		3.751
80	.021		.162		.016		1.083	
85		.137		.310		.032		4.907
90	.017		.133		.030		.900	
95		.119		.328		.024		3.455
*L.S.D. $P < .05$	Not Sig.	.030	.044	.081	.025	.033	.090	.220
Roots								
Degree F								
40	.005		.035		.013		.118	
45		.022		.237		.041		.337
50	.010		.060		.013		.256	
55		.035		.273		.034		.606
65	.008	.021	.022	.067	.009	.004	.546	1.281
70	.005		.012		.004		.491	
75		.021		.049		.003		1.195
80	.008		.007		.011		.436	
85		.028		.043		.002		1.230
90	.005		.014		.009		.344	
95		.026		.050		.004		.862
*L.S.D. $P < .05$	Not Sig.	Not Sig.	.044	.081	Not Sig.	.033	.090	.220

* Least significant difference.

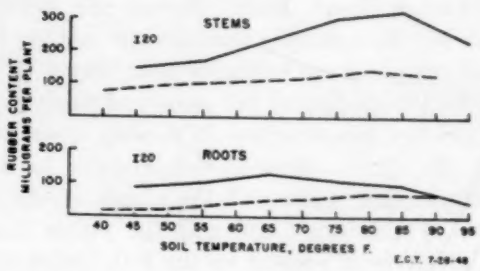

FIG. 6. Grams of rubber in guayule plants grown in different soil temperatures but at an air temperature of 75° F. Data from experiment 1 are shown by broken line; from experiment 2, by solid line. Least significant difference $P < .05$ represented by "I" bar.

larly the suppression of growth at the higher temperatures in experiment 1, the carbohydrate percentage in both experiments showed the same response to increasing soil temperatures. The percentages of resins and rubber, however, showed different responses in the two experiments. In the second experiment, the resin and rubber percentage decreased steadily as the soil temperature was raised but in experiment 1, where some factor seemed to be suppressing the growth of the plants, the percentages of resins and rubber increased as the soil temperature was raised above 65°. Thus, it would seem that the carbohydrate contents of the plants are influenced primarily by the soil temperature, whereas other factors may readily counteract the effect of the soil temperature on the resins and rubber content of guayule plants. It can also be concluded that high soil temperatures *per se* will not prevent rubber accumulation in the guayule plant from the standpoint of either percentage or total yield, but that their effect will vary with other growth conditions imposed on the plants. It would seem that low soil temperature below 65° F will always favor an increase in rubber and carbohydrate percentages.

When actively growing guayule plants are subjected to conditions that check their vegetative growth, as high or low temperature (**2, 18**), drought (**1, 10**), reduction of available essential elements as nitrogen and phosphorus (**3, 12**), and even boron (**13**), rubber accumulates rapidly. BONNER and GALSTON (**5**) have stated that it would seem that rubber accumulation occurs particularly under conditions favorable for assimilation but unfavorable for excessive vegetative growth. The experiments reported here seem to emphasize this point. The air temperatures surrounding the leaves were favorable for photosynthesis, while the low soil temperatures (below 65° F) were unfavorable for rapid vegetative growth and the rubber percentage was higher in the low temperature in both experiments. Soil temperatures above 65° were favorable for vegetative growth and where no growth-suppressing factor was present (experiment 2) the rubber percentage decreased, but where such a factor was present (experiment 1) the rubber percentage increased.

In these experiments, regardless of soil temperature, the air temperature was the same yet the percentages of the various constituents in the tops varied with changes in soil temperature almost exactly as they did in the roots. Of course, to obtain a complete picture of the influences of soil temperature, the air temperature should also be varied. Such experiments are for the future. However, these results do show that, regardless of air temperature, soil temperatures will have a marked influence on the gowth and chemical composition of the above-ground portion of the plants.

With the exception of the water-soluble carbohydrates in the roots of both experiments and the stems of experiment 1, the grams per plant of the various constituents more closely followed the trend in dry weight of

UNIVERSITY OF MICHIGAN LIBRARIES

the plant part than the percentage of the dry weight they made up. In other words, the yield per plant under the conditions of these experiments depended more on the effect of the soil temperature on the total dry weight of the plant than on the percentage composition of the compound under consideration. Thus, the highest total yield of rubber occurred in both experiments in the plants grown in the next to the highest soil temperature or at 80° and 85° F, even though in experiment 2 these plants had a relatively low rubber percentage and in experiment 1 they had a relatively high one. This is important from the standpoint of rubber yields as it indicates that, other conditions being equal, the fairly high soil temperatures will result in the greatest production of rubber.

Summary

1. Two experiments are reported in which guayule plants were grown in a constant air temperature of about 76°, but in soil temperature varying from 40° to 95° F. The soil of the first experiment produced a very rapid initial but a reduced later rate of growth; that of the second produced a fairly rapid rate of growth throughout.

2. In both experiments, the maximum dry weight of the above-ground parts of the plants occurred at a soil temperature of 80° to 85° F and of the roots at a soil temperature of 65° F, while the lowest dry weight of all parts of the plants occurred at 40° to 45° F.

3. In experiment 1, the difference, between the highest and lowest dry weights at the different soil temperatures was proportionately very much less than between those of experiment 2, indicating that some factor such as soil, or condition associated with the year of the experiment, was limiting the growth of these plants so that growth response to soil temperature could not be expressed as it was in experiment 2.

4. The percentage of free sugars, levulins, and inulin in both experiments dropped rapidly as the soil temperature was increased to 65° F, but no further change occurred as the soil temperature was increased to 95° F.

5. The percentage of rubber and resin in the stems and roots in both experiments decreased as the soil temperature was raised from 40° to 65° F, and in the plants of experiment 2 continued to decrease with further increases in soil temperature up to 95° F. In experiment 1, however, where the growth seemed to be depressed, the resins and rubber percentages increased as the soil temperature was raised from 65° to 90° F. It is concluded that low soil temperatures will tend to increase the rubber percentage in guayule plants but that high soil temperatures *per se* will not necessarily mean a low rubber percentage.

6. The yield of rubber on a grams-per-plant basis was associated with the dry weight of the plant rather than the percentage content. The highest yield was obtained from the plants in the 80° to 85° soil temperatures.

7. Although the different treatments were actually applied only to the roots of the plants, both the roots and the top were affected in the same manner so far as the percentage composition of the various compounds determined was concerned.

U. S. NATURAL RUBBER RESEARCH STATION
U. S. DEPARTMENT OF AGRICULTURE
SALINAS, CALIFORNIA

LITERATURE CITED

1. BENEDICT, H. M., MCRARY, W. L., and SLATTERY, M. C. Responses of guayule to alternating periods of low and high moisture stresses. Bot. Gaz. **108**: 535–549. 1947.
2. BONNER, J. Effects of temperature on rubber accumulation by the guayule plant. Bot. Gaz. **105**: 233–243. 1943.
3. BONNER, J. Effect of varying nutritional treatments on growth and rubber accumulation in guayule. Bot. Gaz. **105**: 352–363. 1944.
4. BONNER, J. Further observations on the response of guayule to temperature. Report Emergency Rubber Project. Feb. 1944.
5. BONNER, J., and GALSTON, A. W. The physiology and biochemistry of rubber formation in plants. Bot. Rev. **13**: 543–596. 1947.
6. BROYER, T. C. Observations on the growth of guayule under greenhouse conditions. California Agric. Expt. Station Unnumb. Pub., 16 pp. 1945 (Processed).
7. CAMPBELL, W. A., and PRESLEY, J. T. Design for constant temperature tanks. Phytopathology **35**: 213–216. 1945.
8. ERICKSON, L. C., and SMITH, P. F. Studies on handling and transplanting guayule nursery stock. U. S. Dept. of Agric. Tech. Bull. No. 924. 1947.
9. HAYES, H. K., and IMMER, F. R. Methods of plant breeding. McGraw-Hill Co., New York. 1942.
10. HUNTER, A. S., and KELLEY, O. J. The growth and rubber content of guayule as affected by variations in soil moisture stresses. Jour. Amer. Soc. Agron. **38**: 118–134. 1946.
11. MCRARY, W. L., and SLATTERY, M. C. The colorimetric determination of pentoses and pentosans. Archives of Biochem. **6**: 151–156. 1945.
12. MITCHELL, J. W., WHITING, A. G., and BENEDICT, H. M. Effect of light intensity and nutrient supply on growth and production of rubber and seeds of guayule. Bot. Gaz. **106**: 83–95. 1944.
13. MITCHELL, J. W., BENEDICT, H. M., and WHITING, A. G. Growth, rubber storage, and seed production of guayule as affected by boron supply. Bot. Gaz. **106**: 148–157. 1944.
14. TRAUB, H. P. Concerning the function of rubber hydrocarbon

UNIVERSITY OF MICHIGAN LIBRARIES

(caoutchouc) in the guayule plant, *Parthenium argentatum* A. Gray. Plant Physiol. **21**: 425–444. 1946.

15. Traub, H. P. Rapid photometric methods for determining rubber and resins in guayule tissue and rubber in crude-rubber products. U. S. Dept. of Agric. Tech. Bull. No. 920. 1946.
16. Traub, H. P. Levulins and inulin in guayule, *Parthenium argentatum* A. Gray. Plant Physiol. **22**: 77–87. 1947.
17. Traub, H. P., and Slattery, M. C. Analysis of levulins, inulin and monosaccharides in guayule. Bot. Gaz. **108**: 295–299. 1946.
18. Whitehead, M. R., and Mitchell, J. W. Effects of nutrient, photoperiod, and night temperature on the development of guayule seeds. Bot. Gaz. **105**: 14–24. 1943.

THE ORTHO EFFECT IN PLANT GROWTH-REGULATORS

CORWIN HANSCH AND ROBERT M. MUIR

Received March 10, 1950

Introduction

In an earlier publication (**2**) a comparison was made of the growth regulating activites of various compounds as indicated by the elongation of sections of Avena coleoptiles. From this comparison it was seen that the substitution of various halogen atoms in the phenoxyacetic acid (POA) structure increased its effect on elongation except when both ortho positions were occupied. Also, substitution of methyl groups in the 2, 4, and 6 positions destroyed the activity of both the POA and the phenylacetic acid (PAA) structures. It was proposed that these compounds require a free or potentially free ortho position to effect cell elongation. This paper reports additional evidence for this ortho effect.

Materials and methods

Test plants were grown and used exactly as described previously (**2**). The data are expressed as differences in the average percentage elongation of 15 sections growing in 25 ml. of the solution of the chemical and in distilled water; thus comparisons with previous tests can be made directly.

Three of the compounds studied in this investigation have not been reported in the literature. Following is a description of the methods used for their preparation and some of their physical properties.

2,6,-DIBROMOPHENOXYACETIC ACID.—In 50 ml. of absolute methanol were placed 2.5 g. of 2,6-dibromophenol (obtained from Eastman Kodak), 2.3 ml. of ethylbromoacetate and 0.6 g. of anhydrous sodium methylate. This mixture was refluxed for seven hours. It was then cooled and an equal volume of 10% sodium hydroxide solution was added to it. This mixture was extracted with ether. The ether was then evaporated and the residual ethyl 2,6-dibromophenoxyacetate hydrolyzed for two hours with 50 cc. of 10% sodium hydroxide. Acidification of this solution with hydrochloric acid precipitated 1.4 g. product, m.p. 142–144°. After crystallization from ethanol and then benzene, the pure white crystals melted at 152–153.5° corr.

Analysis: Calcd. for $C_8H_6O_3Br_2$: C, 30.97%; H, 1.94%
Found: C, 31.43%; H, 2.18%

2,5-DICHLOROPHENYLHYDRAZONE OF ETHYL LEVULINATE.—2,5-Dichlorophenylhydrazine (1 g.) was dissolved in just enough dilute hydrochloric acid to effect solution. To this solution was added 0.82 g. of ethyl levulinate. The mixture was shaken and allowed to stand overnight. The brown crystals which separated were purified by crystallization from ligroin (b.p. 65–110°) giving 1.1 g. yield, m.p. 75–76° corr. On standing, these tan

UNIVERSITY OF MICHIGAN LIBRARIES

crystals decomposed. It was necessary to do all further work as soon as possible.

Analysis: Calcd. for $C_{13}H_{18}O_2N_2Cl_2$: C, 51.15%; H, 5.87%
Found: C, 51.61%; H, 5.44%

The phenylhydrazone of the above ester could be hydrolyzed by heating with dilute hydrochloric acid to give the 2,5-dichlorophenylhydrazone of levulinic acid, m.p. 132–133° corr.

Analysis: Calcd. for $C_{11}H_{14}O_2N_2Cl_2$: C, 47.65%; H, 5.05%
Found: C, 47.89%; H, 4.61%

This acid was found to have no plant growth activity.

4,7-DICHLORO-2-METHYLINDOLEACETIC ACID.—1.5 g. of the above dichlorophenylhyrazone was thoroughly ground with 6 g. of anhydrous zinc chloride. This mixture was heated at 180–190° for one hour. It was then cooled and decomposed with dilute hydrochloric acid and ether. The ether layer was separated and the ether evaporated. The residue was boiled for three hours with 25 ml. ethanol and 5 g. of potassium hydroxide. After dilution with water, this mixture was extracted with ether. The aqueous layer was acidified wtih acetic acid. The solution was boiled to remove any excess acetic acid and then cooled to 0° C. The precipitate was removed by filtration, dissolved in sodium bicarbonate solution, filtered and then reprecipitated with acetic acid, yield 0.3 g., m.p. 235° dec. This product was crystallized from benzene-ligroin, m.p. 245° dec. corr.

Analysis: Calcd. for $C_{11}H_9O_2NCl_2$: C, 51.17%; H, 3.5%; N, 5.43%
Found: C, 51.07%; H, 4.2%; N, 5.12%

Experimental results

The data of representative tests of the activities of the compounds at various concentrations are found in table I. Compounds 1, 2, 3, 6, 7, and 9 have both ring positions ortho to the position of attachment of the side chain blocked with halogen atoms or methyl groups. These compounds do not bring about elongation of the coleoptile sections although they have the basic structure for activity in this respect. This lack of activity is particularly striking when one compares compounds 1, 2, and 3 with compound 4 (2,4,5-trichlorophenoxyacetic acid) which has activity of the same order as 2,4-dichlorophenoxyacetic acid (2,4-D) and indoleacetic acid. The loss of activity which occurs when the halogen is moved from the 5 position to the 6 position is duplicated by substituting the open 6 position in 2,4-D with a methyl group giving the 2,4-dichloro-6-methyl derivative of POA. Although it seemed unlikely that the 4 position was of any importance, a compound with this position open, 2,6-dibromophenoxyacetic acid, was prepared and found to be inactive as expected. SYNERHOLM and ZIMMERMAN (**4**) found the 2,6-dichloro derivative likewise inactive in the elongation effects they studied.

This ortho effect has been demonstrated also for PAA and its derivatives. PAA is quite active but the 2,4,6-trimethyl derivative is inactive in in-

ducing cell elongation. The data in table I show that γ-phenylbutyric acid is active but 2,4,6-trimethyl-γ-phenylbutyric acid is inactive and about 10 times more toxic.

In a private communication Professor K. V. Thimann of Harvard University has informed us that he finds samples of the 2,4,6-trichloro, bromo, and methyl derivatives of POA submitted to him by us, to be virtually inactive in the pea test thus confirming the results of the Avena test.

The extension of the ortho effect to the substituted indoleacetic acids is of considerable interest. It may be seen in table I that 2-methylindoleacetic acid induces cell elongation although its activity is less than that of

TABLE I

PERCENTAGE ELONGATION OF SECTIONS OF COLEOPTILES IN SOLUTIONS OF ORGANIC COMPOUNDS AS COMPARED WITH PERCENTAGE ELONGATION IN WATER

	Molar concentrations $\times 10^{-4}$							
	2.0	1.0	0.5	0.1	0.05	0.01	0.005	0.001
1. *2,4,6-Trichlorophenoxyacetic acid		−11	+ 4	0	0	0		0
2. *2,4,6-Tribromophenoxyacetic acid		−11	− 3	0	0	0		0
3. 2,4-Dichloro-6-methylphenoxyacetic acid	−19	−14	+ 6	0	0	0	0	
4. 2,4,5-Trichlorophenoxyacetic acid		− 2	+16	+31	+28	+26	+18	+8
5. *2,4-Dichlorophenoxyacetic acid	− 8	+10	+28	+30	+31	+24	+21	+7
6. 2,6-Dibromophenoxyacetic acid	−13	0	0	0	0	0	0	
7. 2,4,6-Trimethyl-γ-phenylbutyric acid	−23	−19	− 5	− 3	0	0	0	
8. *γ-Phenylbutyric acid	−11	+13	+16	+13	+ 8			
9. 4,7-Dichloro-2-methylindoleacetic acid	−19	+ 6	+12	0		0		0
10. *Indoleacetic acid	+23	+38	+36	+36	+29	+25		0
11. *2-Methylindoleacetic acid	+10	+32	+29	+16	+ 8	0	0	

* These data previously reported (2).

indoleacetic acid. However, when the 4 position is blocked with a halogen atom, as is the case with 4,7-dichloro-2-methylindoleacetic acid, the activity of the molecule is almost completely destroyed. Thus in their effects on the activity of the molecules in cell elongation the 2 and 4 positions in the indole nucleus may be compared with the 2 and 6 positions in POA and PAA. STEVENS and FOX (3) have prepared a number of 2-methylindoleacetic acids with substituents on the benzene ring of the indole nucleus. They report these derivatives to be active in the pea test. None of the compounds which they reported have substituents in both the 2 and 4 positions however.

UNIVERSITY OF MICHIGAN LIBRARIES

Discussion

Without exception the compounds tested in these investigations have demonstrated the existence of an ortho effect in their activity as growth regulators. Compounds which do not have a free or potentially free ortho position do not induce cell elongation. The importance of the ortho position seems best explained by assuming that substituents present in both ortho positions prevent the reaction of the growth regulator with a plant substrate (**2**) . There are two ways in which such groups might function to block such a reaction. First, by blocking a reaction at the ortho position, or second, by steric hindrance of a reaction which might take place through a side chain. In considering the second of the above postulates, it is unlikely that groups in the ortho position would interfere sterically with reactions taking place on the side chain beyond the first two atoms attached to the ring. It has been shown (1) that although diorthosubstituted benzoic acids are extremely difficult to esterify by the usual methods, substitution of a CH_2 group between the carboxyl group and the benzene ring circumvents the influence of the ortho substituents. Thus it seems unlikely that the ortho substituents in the molecules reported in this paper (table I, nos. 1, 2, 3, 6, 7, and 9) should have any important influence on the reaction of the carboxyl group with a substrate. Certainly in the case of 2,4,6-trimethyl-γ-phenylbutyric acid, steric hindrance of the carboxyl group by the methyl groups would be impossible, yet the compound is completely inactive. This leads one to the conclusion that either an ortho position or the first atom in the side chain is involved in the reaction of the growth regulator with a substrate.

That it is the first atom in the side chain which is involved in the reaction seems unlikely when one considers the great variety of units which may constitute this first position (O, NH, S, CH_2, CH=) in molecules which have significant activity with respect to cell elongation. Thus, on the basis of the information available at present, it is most reasonable to assume that the growth regulators are involved in a chemical reaction with a plant substrate through one of the ortho positions. If this assumption is true, then the effects of substituents on the ring should be very important in governing the rate of reaction at the ortho points. An investigation of different electronic characteristics should give a clue as to the nature of the reaction involved. Preliminary data gathered on this problem indicate that, other things being equal, electrophilic groups para to an open ortho position increase the effect of the phenoxyacetic acids, while nucleophilic groups para to an ortho position decrease the effect of the acids in cell elongation. Additional data are required to establish this relationship and a number of other compounds are being tested to evaluate this postulate.

Summary

Further evidence is presented for the blocking of plant growth activity by substitution of both ortho positions in phenoxyacetic and phenylbutyric

acids. This same effect is noted for an indoleacetic acid with the 2 and 4 positions substituted. The evidence is interpreted to mean that plant growth regulators of the above types react with a plant substrate through an ortho position.

CHEMISTRY DEPARTMENT
POMONA COLLEGE
CLAREMONT, CALIFORNIA
AND
BOTANY DEPARTMENT
STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA

LITERATURE CITED

1. MEYER, V., and SUDBOROUGH, J. J. Das Gesetz der Esterbildung aromatischer Säuren. Berichte. **27**: 1580–1592. 1894.
2. MUIR, R. M., HANSCH, C. and GALLUP, A. H. Growth regulation by organic compounds. Plant Physiol. **24**: 359–366. 1949.
3. STEVENS, F. J. and FOX, S. W. Amino acid conversion products. IV. Some substituted 3-indoleacetic acids and some substituted phenylhydrazones of B-formylpropionic acid. Jour. Amer. Chem. Soc. **70**: 2263–2265. 1948.
4. SYNERHOLM, M. E. and ZIMMERMAN, P. W. The preparation of some substituted phenoxy alkyl carboxylic acids and their properties as growth substances. Contr. Boyce Thompson Inst. **14**: 91–103. 1945.

UNIVERSITY OF MICHIGAN LIBRARIES

THE EFFECT OF NITROGEN AND POTASSIUM LEVELS IN TUNG SEEDLINGS ON GROWTH, APPARENT PHOTOSYNTHESIS, AND CARBOHYDRATE COMPOSITION

A. J. Loustalot,[1] S. G. Gilbert, and M. Drosdoff
(WITH THREE FIGURES)

Received March 17, 1950

Introduction

Nitrogen and potassium are the two fertilizer elements that are needed in largest amounts in most tung orchards of the southern United States. Supplying these elements at the optimum rates and in the proper ratios is an important nutritional problem. Leaf analysis has proved to be a valuable criterion of the nutritional status of tung trees, and, as more basic information on the relation of mineral composition to physiological processes and biochemical constituents is accumulated, it should become even more useful. For example, it would be helpful to know the effect of certain concentrations and ratios of nitrogen and potassium in the leaves on the rate of photosynthesis, which in turn must be intimately related to growth, productivity, and storage and utilization of carbohydrates.

The sand-culture technique seemed the best approach to this problem since it afforded a high degree of precision per number of plants, together with greatest ease of control and manipulation. The usual procedure in water or sand cultures is to grow plants in solutions in which the nutrient salts are maintained at definite levels and ratios, and subsequently to make chemical and physiological tests on the plants and on residual or effluent nutrient solutions. In the present study an attempt was made to develop rather specific differences in levels of minerals in the leaves. The composition of representative plants was determined from time to time and the nutrient solutions were adjusted as necessary to produce as nearly as possible the desired mineral concentrations in the plant tissue.

The objectives of this experiment were (1) to grow plants containing three levels of nitrogen and potassium in factorial combinations, while maintaining other elements at optimum or above deficiency concentrations, and (2) to determine the effect of these nutritional variations in the plant on growth, apparent photosynthesis, and chemical composition.

Materials, method, and procedure

General procedure.—On July 17, 1944, selected seedlings from open-pollinated seed of L-2 and L-14 clones were transplanted from the nursery bed to 4-gallon-capacity glazed jars containing medium-coarse sand. The

[1] Now assistant director, Federal Experiment Station, Office of Experiment Stations, Mayaguez, Puerto Rico.

seedlings were two months old and about 12 inches tall with six to nine leaves when transplanted. The holes at the bottoms of the jars were covered with glass wool, and drainage was facilitated by slight inclination of the jars. A total of 66 jars were planted as follows: 30 with one seedling each; 30 with five seedlings each; six with two seedlings each. A complete nutrient solution was supplied daily to all plants for a period of five weeks, ending on August 22. On that date, the 12 seedlings from the six jars of two each were harvested for mineral and carbohydrate analysis and the differential treatments were started. Thereafter, at intervals of about three weeks, one or two representative seedlings were harvested from each of the pots originally containing five plants, for mineral and carbohydrate analysis.[2]

DESIGN OF EXPERIMENT.—A 3 x 3 factorial design was used with three levels each of nitrogen and potassium. Each treatment was replicated six times. The nine jars in each replication were arranged in a single north-south row, the six replications occupying benches side by side in an open area. The treatments were randomized within each replication. Exposure of all the plants to light was fairly uniform, particularly during the time photosynthesis measurements were being made, when all seedlings received the maximum light available.

LEVELS OF POTASSIUM AND NITROGEN DESIRED IN THE LEAVES.—The percentages of potassium desired in the leaves for high, medium, and low levels were 1.2, 0.8, and 0.4%, respectively. Extensive analyses of leaves taken from widely scattered orchards with different soil types and culture indicate that these levels are representative of the range normally found in the field. Leaves containing in the neighborhood of 0.4% potassium usually show definite deficiency symptoms, depending somewhat on the particular conditions, time of sampling, balance of other elements, and other factors. It has also been found that trees grow well and appear healthy with 0.8% potassium in the leaves; and this percentage may be considered an average figure for most healthy orchards. Data obtained in the course of other experiments indicated that increasing the potassium content of the leaves above 0.8% might be effective in increasing growth and productivity; and consequently a level of 1.2% K was adopted as the highest level and one that might induce optimum growth. Only under special conditions has more than 1.2% K been found in tung leaves in the field.

The percentages of nitrogen desired in the leaves for high, medium, and low levels were 2.50, 1.75, and 1.25, respectively. Definite deficiency symptoms have been observed in the field with leaves containing around 1.25% nitrogen; consequently this was taken as the lowest figure. On the other hand, a percentage of 2.5 nitrogen in the leaves has been found to be associated with the best growth and fruiting conditions. An intermediate fig-

[2] Data from plants grown one to a jar and five to a jar were recorded separately to determine if there was any difference in response or composition. Analysis of the data showed no consistent difference in growth or composition between the two types of plants.

UNIVERSITY OF MICHIGAN LIBRARIES

ure of 1.75% was thought to be a sub-optimum level, although fair growth has been observed with trees at this level.

NUTRIENT SOLUTIONS.—Water used in making up the nutrient solutions was de-ionized by running tap water through two Amberlite towers (cation exchanger and anion exchanger) connected in series (8). The variations in concentration of different nutrients necessary to attain the desired nutritional levels within the plant are set forth in detail in table I. These modifications were made in accordance with leaf analyses usually completed within about a week after each sampling date, thus permitting close control of the nutrient levels within the leaf. As a rule, one liter of the nutrient solution was applied in the morning and one in the late afternoon. This was done to maintain the concentration of nutrient salts approximately at the level at which they were applied and to minimize marked changes in pH which normally result from unequal absorption of ions by the plants. The various solutions were adjusted to a pH of 5.2 with sulphuric acid and constant check was made on the pH of the effluent from jars in each treatment. Adjustments in the initial acidity of the various solutions were made when necessary.

The concentrations of calcium and of magnesium in all of the nutrient solutions were unchanged through the experiment (232 p.p.m. Ca and 48 p.p.m. Mg), but the levels of these elements in the leaves of the plants of the various treatments varied somewhat. At the last sampling date, magnesium ranged from 0.28 to 0.72% and calcium from 2.10 to 2.84, with one exception where the percentage of calcium was only 1.50. The calcium and magnesium tended to be negatively correlated with the level of potassium in the leaves. The inverse relationship between potassium and magnesium is found whenever variations in potassium supply occur. A much higher level of magnesium is usually found in the leaves from potassium-deficient tung orchards than in the leaves of trees with an adequate potassium supply, even though soil analyses do not indicate any difference in the amount of available magnesium. The same relationship is found in apple trees (1). The relationship between calcium and potassium is similar but is not so pronounced.

At the last sampling date, the phosphorus content of the leaves ranged from 0.19 to 0.36% and there was some indication of a negative correlation between the concentrations of potassium and phosphorus. Earlier in the experiment, when phosphorus was the same in all solutions, the phosphorus tended to be high in tissues when the nitrogen was high, and low when the nitrogen was low. However, this trend was partially reversed when the nitrogen in the N_3 solution was reduced by half on September 18. In general, the percentage of phosphorus in the leaves of plants in all treatments was well above the usual concentration found in normal trees in the field (table II). It is not known what effect such fluctuations as existed would have on the functions of the plants.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS AT DIFFERENT PERIODS DURING THE EXPERIMENT,* 1944

TREATMENTS AND COMPOSITION NUMBER		AUG. 22 TO SEPT. 4			SEPT. 4 TO SEPT. 18			SEPT. 18 TO OCT. 3			OCT. 3 TO OCT. 27		
		N	P	K	N	P	K	N	P	K	N	P	K
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
N_1K_1	1	9	27	3	0	27	0	0	27	0	0	27	0
N_1K_2	2	9	27	27	0	27	7	0	27	0	0	27	0
N_1K_3	3	9	27	81	0	27	40	0	27	40	0	27	40
N_2K_1	4	81	27	3	81	27	0	0	27	0	0	27	0
N_2K_2	5	81	27	27	81	27	7	0	27	0	0	27	5
N_2K_3	6	81	27	81	81	27	40	0	27	20	0	27	20
N_3K_1	7	162	27	3	162	27	0	81	14	0	81	14	0
N_3K_2	8	162	27	27	162	27	7	81	14	0	81	14	0
N_3K_3	9	162	27	81	162	27	40	81	14	7	81	14	3

SOURCE OF NUTRIENT ELEMENTS

Major Elements	Minor Elements
Nitrogen from $Ca(No_3)_2 \cdot 4\ H_2O$	0.50 to 3 p.p.m. Fe from $FeSO_4 \cdot 7H_2O$
Phosphorus from $K\ H_2PO_4$ and NaH_2PO_4	0.50 p.p.m. Mn from $MnSO_4 \cdot 2\ H_2O$
Potassium from $K\ H_2PO_4$ and K_2SO_4	0.10 p.p.m. B from H_3BO_3
Calcium from $Ca(NO_3)_2 \cdot 4\ H_2O$ and $CaSO_4$	0.10 p.p.m. Zn from $ZnSO_4 \cdot 7H_2O$
Magnesium from $MgSO_4 \cdot 7H_2O$	0.05 p.p.m. Cu from $CuSO_4 \cdot 5H_2O$
Sulphur from SO_4 salts	

* Mg was supplied at 48 p.p.m. and Ca at 232 p.p.m. in all solutions.

UNIVERSITY OF MICHIGAN LIBRARIES

TABLE II

AVERAGE MINERAL COMPOSITION OF LEAVES ON DIFFERENT SAMPLING DATES (DRY MATTER BASIS). EACH ENTRY IS THE AVERAGE OF DETERMINATIONS ON TWO SAMPLES OF THREE PLANTS EACH.

CONSTITUENT	DATE OF SAMPLING	TREATMENTS								
		N_1K_1	N_1K_2	N_1K_3	N_2K_1	N_2K_2	N_2K_3	N_3K_1	N_3K_2	N_3K_3
		%	%	%	%	%	%	%	%	%
Nitrogen	August 22	2.64	2.64	2.64	2.64	2.64	2.64	2.64	2.64	2.64
	September 11	2.12	1.91	1.93	3.08	3.08	3.43	3.29	3.27	3.44
	October 3	1.59	1.43	1.74	2.44	2.42	2.32	2.79	2.90	3.01
	October 27	1.39	1.41	1.35	1.84	1.89	2.14	2.48	2.80	2.51
Potassium	August 22	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
	September 11	0.77	1.25	1.43	0.79	1.50	1.80	0.83	1.40	1.81
	October 3	0.64	1.11	1.31	0.50	0.90	1.50	0.51	1.06	1.53
	October 27	0.57	0.96	1.28	0.46	1.05	1.51	0.47	0.80	1.42
Phosphorus	August 22	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
	September 11	0.17	0.15	0.16	0.22	0.24	0.24	0.22	0.22	0.23
	October 3	0.18	0.16	0.18	0.18	0.20	0.22	0.23	0.21	0.21
	October 27	0.36	0.29	0.29	0.30	0.24	0.24	0.25	0.24	0.19
Calcium	August 22	2.55	2.55	2.55	2.55	2.55	2.55	2.55	2.55	2.55
	September 11	2.51	2.32	2.12	2.45	2.12	2.16	2.42	2.42	2.17
	October 3	2.68	2.18	2.25	2.37	2.16	2.04	2.70	2.25	2.12
	October 27	2.82	2.60	2.44	2.80	2.12	2.10	2.84	2.62	1.50
Magnesium	August 22	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	September 11	0.54	0.41	0.47	0.50	0.46	0.41	0.52	0.48	0.39
	October 3	0.57	0.49	0.49	0.54	0.43	0.34	0.56	0.43	0.38
	October 27	0.72	0.54	0.47	0.67	0.44	0.28	0.55	0.46	0.32
Ash	September 11	10.78	11.03	10.56	10.76	10.78	10.90	10.14	11.22	11.19
	October 3	10.72	10.08	10.78	9.40	9.52	9.90	10.64	10.38	10.61
	October 27	11.25	10.51	11.28	10.18	9.19	9.80	10.61	9.82	8.90

Shortly after the treatments started, the plants growing in the high nitrogen solutions began to show symptoms of iron deficiency, although the amount of iron supplied was the same for all treatments. The pH of the effluent from the N_3 treatments was found to be above 6, which was undoubtedly a factor in rendering the iron in the solution unavailable to the plants. This relatively high pH was probably due to increased absorption of anions such as nitrate and phosphate over that of cations like calcium and potassium, thus leaving a basic residue in the nutrient solution. This difficulty was brought under control when the treatment was altered by lowering the pH of the initial solution to 4.8 with sulphuric acid, reducing the concentration of nitrate ions in the nutrient solution, spraying the plants with a dilute solution of iron sulphate (50 p.p.m.), and increasing the iron content of the nutrient solution to 3 p.p.m.

SAMPLING METHODS AND GROWTH MEASUREMENTS.—At the time differential treatments were started (August 22), 12 plants were harvested from six jars each of which contained two plants. These were composited for chemical analysis into two groups of six plants each. On September 11 and on October 3, six plants were harvested from each treatment from jars that originally contained five seedlings. After the October 3 sampling, each jar contained a single seedling and these were harvested on October 27. The six plants harvested from each treatment were composited into groups of three each and analyzed as two replications for total ash, nitrogen, calcium, magnesium, and phosphorus as outlined by DROSDOFF and PAINTER (**3**). Leaves, stems, and roots were analyzed separately. Analyses were also made for reducing sugars, nonreducing sugars, starch, and polysaccharides. The carbohydrate methods were essentially those described by SELL *et al.* (**10**). The principal modification was in the use of oven-dried material instead of alcohol-preserved material. The composite plant materials were heated in a forced-draft tray oven at 105° C for 10 minutes and then dried to constant weight at 70° C. The leaves and fibrous roots were dried in about three hours, the wood tissue requiring a longer period.

APPARENT PHOTOSYNTHESIS.—The rate of apparent photosynthesis was determined daily, August 22 to October 27, inclusive, using the HEINICKE-HOFFMAN (**7**) procedure. Eleven carbon dioxide absorption towers were used for the determination of CO_2. Two of these served as air checks and the remaining nine were connected to assimilation chambers (**6**) each attached to a single leaf on a plant in each of the nine treatments. Each day the assimilation chambers were attached to plants of a different replication. Thus, at the end of six days, data were obtained on all the replications of each treatment. The photosynthesis measurements were usually made twice daily, in the morning from 9:30 a.m. to 1:00 p.m., and in the afternoon from 1:30 to 4:30 p.m.

Growth measurements, which included height, diameter of trunk, number of leaves, and leaf area, were obtained from all the plants in the experiments

UNIVERSITY OF MICHIGAN LIBRARIES

on the four sampling dates. In addition, fresh and dry weights of the individuals harvested on each date were also obtained.

Results

Analysis of variance showed there was no significant interaction between N and K on the dry weight. Accordingly, the total dry weight of the plants in each nitrogen level, regardless of potassium level, and the total dry weight of the plants in each potassium level, regardless of nitrogen level, are presented graphically in figure 1, in which each point represents the

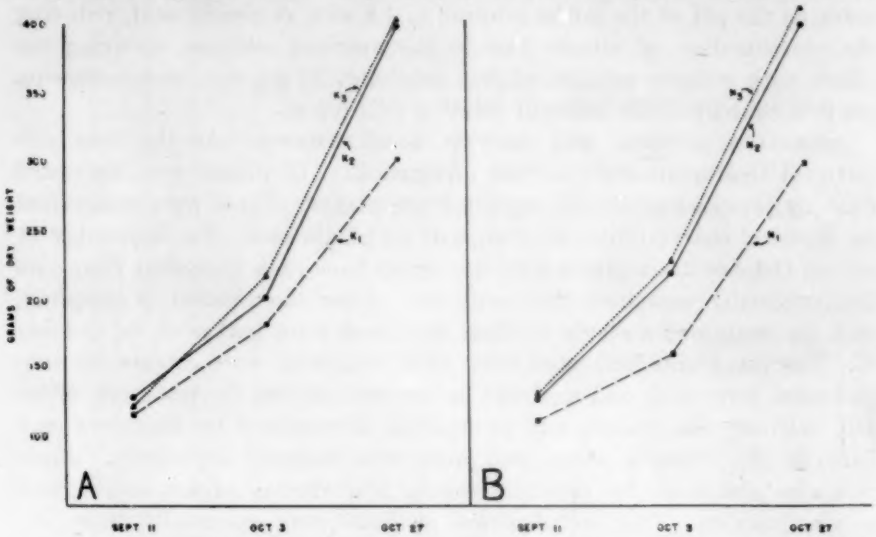

FIG. 1. Effect of three levels of potassium and three levels of nitrogen on the dry weight of tung seedlings. *A*, effect of potassium; *B*, effect of nitrogen. Each level of nitrogen or potassium is represented by the total dry weight of 18 plants on each sampling date.

total weight of 18 plants. The low level of nitrogen had the earliest and most profound effect in retarding the dry weight development of the seedlings. The dry weights of the N_2 and K_2 plants throughout the experiment were not appreciably different from those of the N_3 and K_3 plants. Statistical analysis of the growth data indicated that the effect of nitrogen on dry weight of the plant at the October 3 sampling date was significant at the 5% level and attained significance at the 1% level at the last sampling date. On the other hand, the effect of potassium on dry weight attained significance only at the last harvest. Both N and K had a highly significant effect on trunk diameter and plant height by October 27. However, there was no appreciable difference in diameter and height between plants at the second and third levels of N or between those at the second and third levels of K.

The data with respect to number and area of leaves at the last sampling date followed a trend similar to that of the other growth measurements; the

N_1 treatment had the smallest number and area of leaves. The leaf area of the N_2 plants was similar to that of the N_3 plants, except at the last sampling date when the N_2 plants had a somewhat smaller leaf area, mainly because some of the basal leaves had abscised. Plants at the K_1 level differed significantly in leaf area from those at the K_2 and K_3 levels, but to a lesser degree than in the case of nitrogen. There was no appreciable difference in leaf area between the K_2 and K_3 levels.

The first visible symptoms of deficiency occurred, as was expected, in the N_1 plants. This was first apparent on September 9, about three weeks after differential treatments had started and two days before the September 11 harvest, which coincided with a depression in the rate of CO_2 assimilation. The leaves of the N_1 plants were lighter green in color than those of N_2 and N_3 plants and there was a definite check in growth. As time went on, they became more chlorotic and by September 20 some of the basal leaves had turned yellow and abscised. This became progressively more pronounced. The K_1 plants were retarded in growth, but at no time during the experiment were characteristic leaf symptoms of potassium deficiency evident in the leaves, although the percentage of K in the leaves declined to 0.46 in the plants of N_2K_1 treatments at the final harvest date.

The leaves of the N_2 plants began to show symptoms of incipient nitrogen deficiency about two weeks before the experiment ended and chemical analysis showed the percentage of nitrogen in the leaves to be similar to that found in the N_1 plants at the time the deficiency symptoms appeared in them.

Apparent photosynthesis.—Since an analysis of variance indicated that there was no significant interaction between nitrogen and potassium on the rate of apparent photosynthesis, only the main effects of these elements will be presented. Nitrogen and potassium each had a significant effect on the rate of CO_2 assimilation. Presented graphically in figures 2 and 3 are the main effects of nitrogen and potassium on the rate of apparent photosynthesis with the corresponding percentages of nitrogen and potassium in the leaves at the four sampling dates.

It will be seen from figure 2 that there was a slight reduction in the rate of carbon dioxide assimilation of N_1 plants, particularly in the afternoon, about two to three weeks after the treatment was started. The average percentage of nitrogen in the leaves of the N_1 plants at this time (September 11) was 1.98. The photosynthesis rate of these plants showed a sharp drop the following week, and was reduced somewhat further during the next two weeks until it was approximately one-half that of the high nitrogen treatment at the time of the October 4 sampling date. The percentage of nitrogen in the leaves at this time was 1.58. The following week the rate of apparent photosynthesis dropped to about 35% of that of the N_3 plants, and that relative rate was maintained more or less closely

UNIVERSITY OF MICHIGAN LIBRARIES

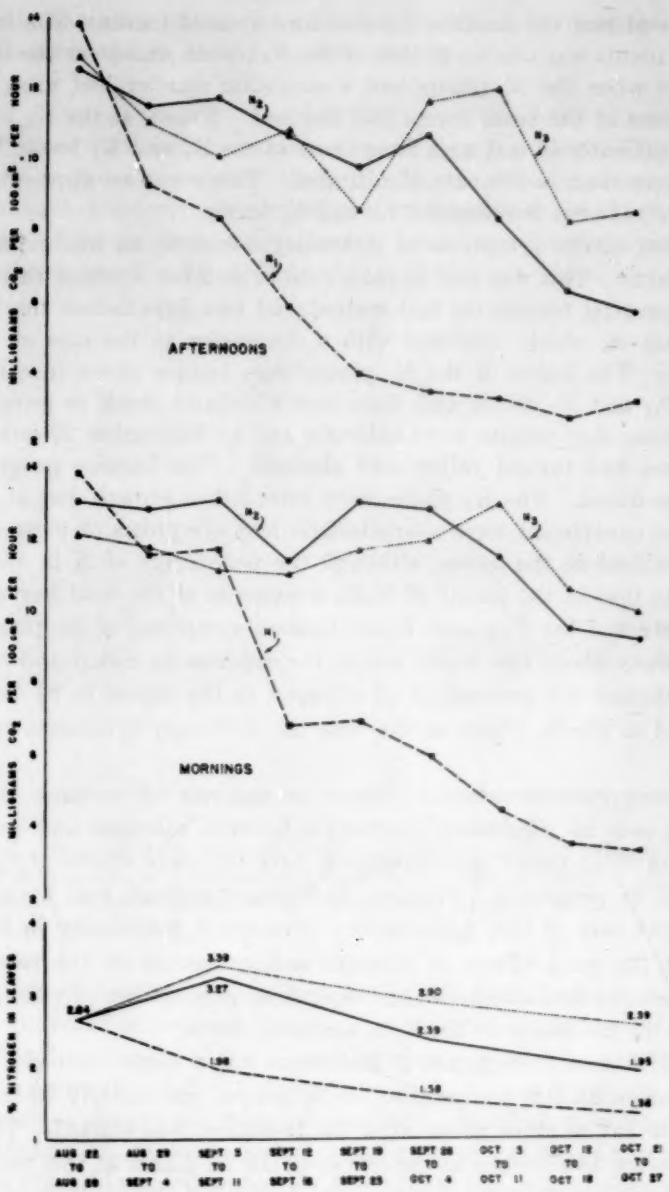

FIG. 2. Effect of three nitrogen levels in the leaves on the photosynthetic activity of tung seedlings. Each point is the average daily rate for any given week—18 determinations per treatment.

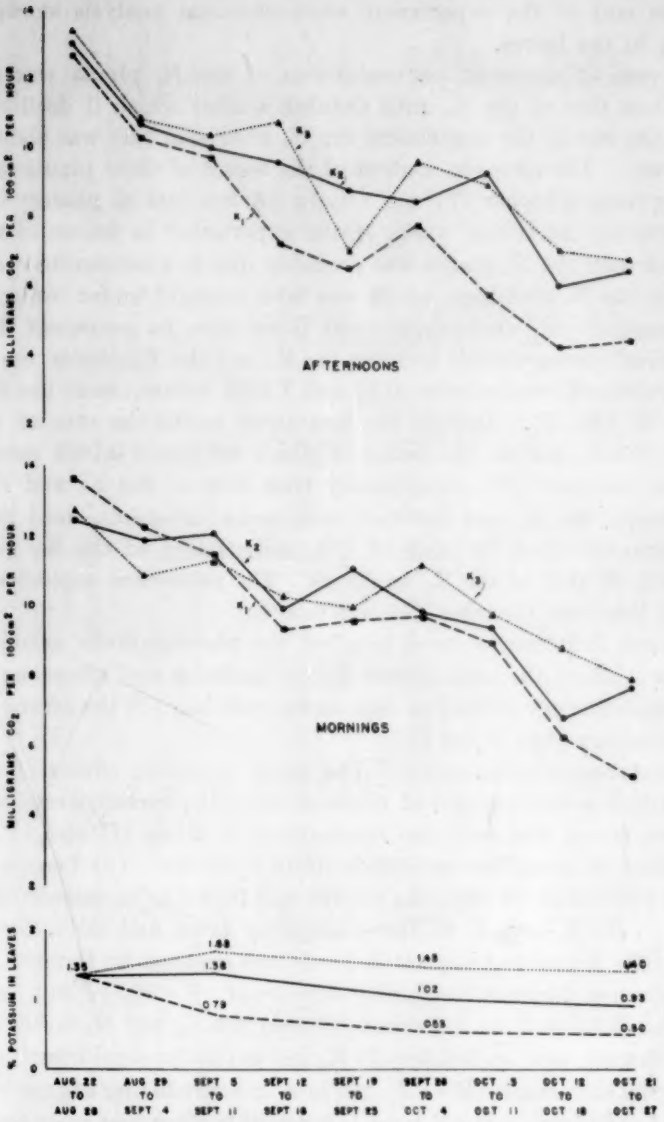

FIG. 3. **Effect of three potassium levels in the leaves on the photosynthetic activity of tung seedlings. Each point is the average daily rate for any given week—18 determinations per treatment.**

UNIVERSITY OF MICHIGAN LIBRARIES

until the end of the experiment when chemical analysis showed 1.38% nitrogen in the leaves.

The rate of apparent photosynthesis of the N_2 plants was somewhat higher than that of the N_3 until October 4 after which it declined gradually; at the end of the experiment the N_2 afternoon rate was about 75% of the N_3 rate. The nitrogen content of the leaves of these plants on the last sampling date (October 27) was 1.95%. A low rate of photosynthesis observed during the initial weeks of the experiment in leaves of the N_3 as compared with the N_2 plants was probably due to a concomitant deficiency of iron in the N_3 seedlings, which was later brought under control.

Throughout the whole experiment there were no consistent differences in apparent photosynthesis between the K_2 and the K_3 plants, the leaves of which contained, respectively, 0.93 and 1.40% potassium at the final date, October 27 (fig. 3). During the first three weeks the rate of photosynthesis in the K_1 plants, the leaves of which contained 0.79% potassium at that time, did not differ significantly from that of the K_2 and K_3 plants. Subsequently the K_1 rate declined, with some variations, until the end of the experiment when the rate of CO_2 assimilation of the K_1 plants was about 65% of that of the K_3 seedlings. The potassium content of the K_1 leaves at this time (October 27) was 0.50%.

Nitrogen deficiency seemed to affect the photosynthetic activity of the seedlings to about the same degree during morning and afternoon, whereas potassium deficiency seemed to depress the rate more in the afternoons than in the mornings (figs. 2 and 3).

CARBOHYDRATE COMPOSITION.—The main treatment effects of the three levels each of potassium and of nitrogen upon the carbohydrate content of the leaves, stems, and roots are summarized in tables III and IV.

1. Effect of potassium on carbohydrate fractions. (a) Leaves. A lowering in percentage of reducing sugars was found to be associated with an increase in the K level at all three sampling dates, and the difference produced by the lowest and highest K levels was greatest on October 27. The overall average decrease was highly significant ($F = 30.5$);[3] but on September 11 and October 3 the difference between the K_2 and K_3 levels was without significance, and on October 27 K_1 did not differ significantly from K_2.

A highly significant ($F = 8.9$) increase in nonreducing sugars was found associated with increase in K level. Again this effect was most pronounced at the last sampling date, corresponding to the period of greatest difference in K levels in the leaves.

No significant main effect of K on the starch content was noted; but there was a highly significant interaction of K with date, and on the last sampling date the starch content of the K_3 leaves was only 1.90% in comparison with 3.23% in the K_1 leaves, a difference of 0.45 percentage units

[3] The F values required for all treatment main effects reported in this paper are: at .05, 4.46; at .01, 8.65; at .001, 18.49.

TABLE III

CARBOHYDRATE COMPOSITION OF TUNG SEEDLINGS AS AFFECTED BY POTASSIUM LEVEL IN THE PLANT (DRY MATTER BASIS). EACH ENTRY IS THE AVERAGE OF DETERMINATIONS OF SIX SAMPLES OF THREE PLANTS EACH

CONSTITUENT	SAMPLING DATES									SIGNIFICANT DIFFERENCE*
	SEPT. 11			OCT. 3			OCT. 27			
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
	%	%	%	%	%	%	%	%	%	
Leaves										
K percentage in leaves	0.79	1.38	1.68	0.55	1.02	1.45	0.50	0.93	1.40	
Reducing sugars	6.35	5.49	5.14	8.23	7.62	7.32	8.57	8.76	6.57	0.65
Nonreducing sugars	1.38	1.51	1.93	0.81	0.65	1.04	1.88	2.77	3.26	0.72
Starch	2.02	1.94	2.18	2.00	2.21	2.66	3.23	2.87	1.90	0.45
Other polysaccharides	5.68	5.79	5.88	6.03	5.07	5.86	5.19	5.01	5.52	0.61
Stems										
K percentage in stems							0.34	0.59	0.86	
Reducing sugars	7.62	5.74	4.70	4.63	4.29	3.29	2.99	2.44	2.71	0.36
Nonreducing sugars	3.54	3.67	2.90	3.99	4.37	4.55	4.31	4.54	4.27	0.72
Starch	6.16	6.55	5.79	12.58	10.86	9.90	10.32	9.92	9.22	1.68
Other polysaccharides	10.53	9.82	9.41	12.47	11.98	12.37	11.32	11.45	10.74	2.35
Roots										
K percentage in roots							0.88	1.66	2.38	
Reducing sugars	6.98	5.79	3.21	6.32	5.58	2.98	7.76	6.18	2.88	2.66
Nonreducing sugars	1.20	1.43	1.52	1.64	2.14	3.06	2.19	3.16	2.99	0.91
Starch	1.57	1.82	1.93	1.69	1.83	1.65	2.26	2.22	2.41	0.56
Other polysaccharides	9.50	7.37	8.05	5.41	8.41	9.20	9.59	10.10	9.70	2.92

* Least mean difference significant at .01 level.

UNIVERSITY OF MICHIGAN LIBRARIES

TABLE IV

CARBOHYDRATE COMPOSITION OF TUNG SEEDLINGS AS AFFECTED BY NITROGEN LEVEL IN THE PLANT (DRY MATTER BASIS). EACH ENTRY IS THE AVERAGE OF DETERMINATIONS ON THREE SAMPLES OF THREE PLANTS EACH

CONSTITUENT	SAMPLING DATES									
	SEPT. 11			OCT. 3			OCT. 27			SIGNIFICANT DIFFERENCE*
	N_1	N_2	N_3	N_1	N_2	N_3	N_1	N_2	N_3	
	%	%	%	%	%	%	%	%	%	
Leaves										
N percentage in leaves	1.98	3.27	3.39	1.58	2.39	2.90	1.38	1.95	2.59	
Reducing sugars	6.49	5.42	5.07	7.88	8.74	6.55	8.58	9.06	7.36	0.65
Nonreducing sugars	1.56	1.36	1.89	0.31	0.73	1.46	1.64	2.25	4.02	0.72
Starch	2.99	1.60	1.54	2.79	2.44	1.64	1.56	3.18	3.26	0.45
Other polysaccharides	6.68	5.48	5.19	6.95	5.37	4.66	5.69	5.40	4.63	0.61
Stems										
N percentage in stems	0.67	1.19	1.26	0.58	0.82	1.23	0.63	0.82	1.28	
Reducing sugars	6.36	6.54	5.16	4.38	4.29	3.62	2.58	2.33	3.23	0.36
Nonreducing sugars	4.92	2.39	2.80	4.46	4.12	3.64	4.14	4.81	4.18	0.72
Starch	10.03	4.09	4.38	15.54	6.86	11.05	10.63	10.02	8.81	1.68
Other polysaccharides	9.99	9.85	10.00	12.05	13.13	11.64	10.49	14.04	8.99	2.35
Roots										
N percentage in roots	1.44	2.57	2.66	1.37	1.93	2.45	1.11	1.48	2.23	
Reducing sugars	7.01	4.13	4.84	5.63	4.68	4.58	5.13	6.67	5.03	2.66
Nonreducing sugars	2.18	1.18	0.70	2.22	3.36	1.26	2.09	3.88	2.37	0.91
Starch	2.39	1.16	1.76	1.62	1.95	1.59	1.95	2.42	2.53	0.56
Other polysaccharides	8.49	7.74	8.69	6.84	9.57	6.62	9.85	9.57	9.97	2.92

* Least mean difference significant at .01 level.

being required for high significance. The effect of K on polysaccharides other than starch was of minor significance.

(b) Stems. The decrease in percentage of reducing sugars with increase in K level was especially marked in the stems at the first sampling date, but was only slight at the last date, when the general level of reducing sugar was low. The nonreducing sugar content fluctuated without significant trend. At all dates starch was lowest at the highest K level, the trend being of high significance. The trend in the other polysaccharides fraction was not significant for stems.

(c) Roots. At all three sampling dates, the reducing sugar content of the roots was consistently depressed with an increase in K level. The percentage of reducing sugars at the K_1 level was approximately twice that of the K_3 level at each of the three sampling dates. The nonreducing sugar percentage tended to increase with an increase in K level at all three dates, just as it did in the leaves.

The starch and other polysaccharides showed no consistent effects of K.

2. Effect of nitrogen levels on carbohydrate fractions. (a) Leaves. The effects of N on reducing and nonreducing sugar percentages in the leaves were similar to those of K, except that they were of a higher order of significance, particularly the increase in nonreducing sugars with an increase in N level. Starch percentage showed a decrease with increase in N at the first two sampling dates, followed by a striking reversal of the trend at the last sampling date.

The effect of increasing N was to lower, in a highly significant manner, the other polysaccharide reserves of the leaves.

(b) Stems. As in the case of K, the effect of increasing N in lowering the percentage of reducing sugars in the stem was most outstanding at the first sampling date; at the last sampling date it was reversed. A similar downward trend was found in the nonreducing sugar and starch content of the stem, the greatest decrease produced by increasing N level being at the first date. The F value of 198.9 for the effect of N on starch content was the largest obtained from this study. An apparent tendency for other polysaccharides to be high at the intermediate level of N attained high statistical significance ($F = 8.9$).

(c) Roots. The reducing sugar content of the roots was highest at the first sampling date at the N_1 level, but neither the main effect of N nor the interaction of N with date attained statistical significance.

A highly significant general downward trend of nonreducing sugars with increase in N was found at the first date, whereas at the third date the highest percentage was at intermediate N. A tendency apparent on September 11 for starch to decrease at high levels of N was reversed on October 27. The data for other polysaccharides showed no consistent trends.

3. Interactions. The most strikingly significant interaction of N with

UNIVERSITY OF MICHIGAN LIBRARIES

K was that of starch content of the leaves. At the low level of N, percentage of starch increased with level of K, while the reverse was true at the intermediate and high levels of N (table V).

TABLE V

STARCH CONTENT OF LEAVES AS AFFECTED BY THE INTERACTION OF NITROGEN WITH POTASSIUM (AVERAGES FOR THREE SAMPLING DATES)

NITROGEN LEVEL	PERCENTAGE OF STARCH IN LEAVES		
	POTASSIUM LEVEL		
	LOW	INTERMEDIATE	HIGH
Low	2.10	2.45	2.80
Intermediate	2.66	2.59	1.97
High	2.49	1.98	1.98

Least difference significant at .01 is 0.36.

In the case of nitrogen, the trends of all constituents differed more widely at the three dates than in the case of K. The starch content of the leaves is an outstanding example of this interaction of level of N with dates ($F = 79.5$, where 7.5 is required for statistical significance at the .001 level). This is in keeping with the fact that at the beginning of the experiment plants at all levels of nitrogen were healthy, while during the last period the plants ranged from severely deficient at N_1 to typical high nitrogen plants at N_3. For example, the general effect of nitrogen in lowering starch content becomes marked by the failure of low-nitrogen plants to continue production of starch. Thus the picture at the first sampling period, when no deficiencies but rather luxury levels prevailed, is often quite different from that at the last date, when deficiency limits for N and K had developed.

Discussion

The reduction in the rate of photosynthesis of the N_1 plants coincided with the first visible evidence of nitrogen-deficiency symptoms in the leaves. Associated with this was a check in growth resulting in reduced dry weight by the September 11 harvest date (fig. 1). Since the nitrogen content of the leaves at this time was 1.98, this value is probably very close to the "critical concentration" at which photosynthesis and growth are affected. It is of interest to note that the rate of photosynthesis of the N_2 seedlings toward the end of the experiment was approximately the same as that of the N_1 plants earlier in the experiment when the nitrogen content was also approximately the same. This is additional evidence that the percentage value of 1.98 is fairly close to the critical level of nitrogen for photosynthesis and growth of young tung seedlings. When the nitrogen content of tung leaves approaches this value, there is insufficient nitrogen available to combine with the carbohydrates to form protein and other plant constituents necessary for growth, and this results in a check in

growth and an accumulation of the carbohydrates. The data at hand do not answer the question as to whether the accumulation of carbohydrates induces the initial reduction in CO_2 assimilation or whether the reduction is due primarily to the failure of some plant constituent, such as chlorophyll, to be formed in sufficient amounts, so that it becomes a limiting factor in the process of photosynthesis.

By October 3 the nitrogen deficiency of the N_1 plants was fairly severe, the percentage of the element in the leaves being 1.58. Linear growth had stopped and gain in dry weight was only about 75% of that made by N_2 and N_3 plants.

The low level of potassium had a less profound influence on reducing the rate of photosynthesis from that of the high level than did the difference in levels of nitrogen. As might be expected, the effect on dry weight was generally of the same order of magnitude as the relative photosynthesis rates, until the last sampling date when the dry weights of the K_1 and N_1 plants were approximately the same.

It will be seen from figure 3 that the rate of CO_2 assimilation of K_1 plants was depressed more in the afternoons than in the mornings. One important factor which might account for this was the higher air temperatures which normally prevailed during the afternoon. If potassium deficiency causes an increase in the rate of respiration, as has been shown by Gregory and Richards (**5**) and by Richards (**9**), then the lower rate of apparent photosynthesis in the afternoon could be attributed to the higher temperature, since a rise in temperature accelerates the rate of respiration more than it does the rate of photosynthesis (**5, 9**). Nitrogen deficiency, on the other hand, affected photosynthesis to about the same degree in the morning as in the afternoon, which indicates that it has no appreciable effect on the rate of respiration, although low N is presumed to be associated with low respiration.

It is clear from the data on carbohydrate composition that the percentage of reducing sugars was almost without exception lowest at the K_3 level and highest at the K_1 level at the three sampling dates and in the three portions of the plant. This effect was particularly marked in the roots. The concentration of starch, one of the important carbohydrate reserves, followed a trend somewhat similar to that of reducing sugars, particularly in the stem, which contained the greater portion of the starch of the tung plant. The effect of K on the nonreducing sugars in the leaves was practically the reverse of its effect on the reducing sugars. On all sampling dates markedly greater amounts of nonreducing sugars were found at the K_3 level than at K_1 or K_2.

The directly opposite effect of K levels on accumulation of the two sugar fractions must be correlated with the facts that rates of photosynthesis and of growth were highest at the high K levels. Assuming first that sucrose is the photosynthetic end product, second that reducing sugar is the prin-

UNIVERSITY OF MICHIGAN LIBRARIES

cipal metabolite utilized in growth and respiration, and third that the rate of inversion of sucrose is relatively slow, sucrose would accumulate at high K levels where the reducing sugar formed would be most rapidly depleted.

If, on the other hand, the hexoses are the first products of photosynthesis, the data would indicate that condensation to sucrose and starch occurs most rapidly at high K levels even though utilization tends to keep the hexoses at a low level. It should be noted that the trend in nonreducing sugars is the same in roots and leaves. Since hexoses are generally assumed to be the translocation form of sugar, the data from stems and roots tend to support the condensation hypothesis.

The effects of N on composition of stems and roots may be interpreted on the basis of relative growth. The top/root ratio is low at low N and high at high N. The products of photosynthesis tended to be utilized in regions of active growth, for example, in stems at high N; and to accumulate as reserves in regions of limited growth, for example, in roots at high N.

A highly significant $N \times K$ interaction on starch content of the leaves was noted. Starch accumulation at low K was greatest at high levels of N. Starch also tended to accumulate in the leaves at low N with high K. Apparently a low level of either element in the presence of a high level of the other causes growth to decline more rapidly than photosynthetic activity. This experiment can be compared with a field experiment involving bearing tung trees deficient in N and K (**4**). In that experiment the effects of nitrogen on carbohydrate accumulation were in general agreement with those of this experiment, especially with regard to the increased sucrose content of the leaves with increased N level.

In the field experiment and in an experiment with tomatoes reported by WALL (**11**), the first stage of K deficiency was associated with high carbohydrate content. In subsequent growth stages the carbohydrate content of the deficient plants decreased to the levels of the K-treated plants. This may explain the failure to obtain any relatively high starch content in the low-K plants until the end of the experiment.

Summary

A study is reported in which three levels of nitrogen and of potassium in factorial combinations were obtained in leaves of tung seedlings growing in sand culture. The percentages of nitrogen desired in the leaves were 2.50, 1.75, and 1.25; those obtained at the last harvest date were 2.59, 1.95, and 1.38. The percentages of potassium desired in the leaves were 1.20, 0.30, and 0.40; those obtained were 1.40, 0.93, 0.50.

When the nitrogen content of the leaves of N_1 plants fell to about 2.00%, a change in color of the foliage was noted, and both rate of apparent photosynthesis and rate of growth declined. When, at a later date, the nitrogen content of the leaves of the N_2 plants approached 2.00%, similar effects were observed. In plants, the leaves of which contained only about 1.60 to 1.40% of nitrogen, photosynthesis was at a very low level, linear growth

stopped, many leaves abscised and increase in dry weight was nearly at a standstill.

Decline of the potassium content of the leaves to about 0.55% failed to produce symptoms in the leaves and reduced the rates of apparent photosynthesis and growth to a lesser extent than did the low levels of nitrogen reported above.

Nitrogen deficiency reduced the rate of apparent photosynthesis to about the same extent morning and afternoon, but potassium deficiency depressed photosynthesis to a greater extent in the afternoon. This may have been due to a tendency of the low level of potassium to increase the rate of respiration during the warmest part of the day.

Although a low level of potassium was associated with a decrease in rate of photosynthesis and of growth, there was a concomitant increase in reducing sugars and decrease in nonreducing sugars, particularly marked in the roots. This may indicate that potassium plays a role in condensing hexose to disaccharide sugars. On the other hand if sucrose is assumed to be the primary product of photosynthesis, it would indicate a decrease in rate of production of the sucrose with rapid inversion to reducing sugars, even though the latter were not rapidly consumed in growth.

A low level of nitrogen in the leaves tended to be associated with low levels of nonreducing sugars and high levels of reducing sugars in the leaves, but the effects on stems and roots were less consistent.

Starch in the leaves appeared to accumulate most rapidly at N_1K_3 and at N_3K_1. Apparently a low level of one of these elements in the presence of a high level of the other checked growth more than photosynthesis, with the result that starch tended to accumulate.

Division of Fruit and Vegetable Crops and Diseases
Bureau of Plant Industry, Soils, and Agricultural Engineering
Agricultural Research Administration
U. S. Department of Agriculture
Gainesville, Florida

LITERATURE CITED

1. Batjer, L. P. and Degman, E. S. Effect of various amounts of nitrogen, potassium, and phosphorus on growth and assimilation in young apple trees. Jour. Agr. Res. **60**: 101–116. 1940.
2. Childers, N. F. The influence of certain nutrients on the photosynthetic activity of apple leaves. Amer. Soc. Hort. Sci. Proc. **35**: 253–254. 1937.
3. Drosdoff, M., and Painter, J. H. A chlorosis and necrosis of tung leaves associated with potassium content. Amer. Soc. Hort. Sci. Proc. **41**: 45–51. 1942.
4. Drosdoff, M., Sell, H. M., and Gilbert, S. G. Some effects of potassium deficiency on the nitrogen metabolism and oil synthesis in the tung tree (*Aleurites fordii*). Plant Physiol. **22**: 538–547. 1947.

UNIVERSITY OF MICHIGAN LIBRARIES

5. Gregory, F. G., and Richards, F. J. Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. Ann. Bot. **43**: 119–161. 1929.
6. Heinicke, A. J. A special air chamber for studying photosynthesis under natural conditions. Science **77**: 516–517, illus. 1933.
7. Heinicke, A. J., and Hoffman, M. B. The rate of photosynthesis of apple leaves under natural conditions. Part I. New York (Cornell) Agric. Expt. Sta. Bul. 577, 32 pp. illus. 1933.
8. Liebig, G. F., Jr., Vanselow, A. P., and Chapman, H. D. The suitability of water purified by synthetic ion-exchange resins for the growing of plants in controlled nutrient solutions. Soil Sci. **55**(5): 371–376. 1943.
9. Richards, F. J. Physiological studies in plant nutrition. III. Further studies of the effect of potash deficiency on the rate of respiration in leaves of barley. Ann. Bot. **46**: 367–388. 1932.
10. Sell, H. M., Johnston, F. A., and Lagasse, F. S. Changes in the chemical composition of the tung fruit and its component parts during development. Jour. Agr. Res. **73**: 319–334. 1946.
11. Wall, M. E. The role of potassium in plants: I. Effect of varying amounts of potassium on nitrogen, carbohydrate, and mineral metabolism in the tomato plant. Soil Science **47**: 143–161. 1939.

MOISTURE ABSORPTION BY PLANTS FROM AN ATMOSPHERE OF HIGH HUMIDITY

E. L. Breazeale, W. T. McGeorge and J. F. Breazeale

(WITH THREE FIGURES)

Received January 31, 1950

The physics of water movement in the soil has been the subject of a great deal of investigation. The role of the plant in soil moisture and soil moisture-plant relationships has been studied less intensively. Under arid and semi-arid conditions plants are often subjected to tensions extending over a wide range of soil moisture percentages, and there is evidence that the plants themselves often contribute to their own survival under moisture stress by absorption and exudation of moisture.

Breazeale (**1**), and Breazeale and Crider (**2**) have shown that a plant can absorb water and transport it from one zone of low tension to one of high tension if a part of its root system is present in both zones. Breazeale and McGeorge (**3**) have shown that this phenomenon can be used to determine the wilting percentage of the soil—that is, the equilibrium occurring when the water-retaining forces of the soil equal the water-absorbing forces of the plant roots.

In the semi-arid southwest summer crops exhibit a distinctly different growth response to rain, which is accompanied by a high humidity, than they do to an irrigation which is accompanied by a low humidity. Winter crops which are grown under more favorable atmospheric conditions with respect to humidity and temperature show less moisture stress than summer-grown crops. It is of interest, in this connection, that along the coast of California, where fogs exist, some crops are grown without irrigation and with only a few inches of rainfall.

The observations of Brierley (**4**) are of interest in this connection. He noted an increase in size of raspberries following a very light shower during which only enough rain had fallen to wet the leaves. The soil was not wetted. He postulated that the increase in size of berries, following such a light shower, might be due to absorption of water by the leaves. In order to study this further, he selected leaves from a large number of different plants and allowed them to wilt after sealing the stem with paraffin. When these wilted leaves were immersed in water they recovered turgidity.

While it is recognized that a low transpiration rate reduces the moisture stress, the question of water intake through the leaves is pertinent. The following experiments were conducted to study water intake through leaves when the above-ground part of the plant is subjected to fog or highly humid atmospheres.

UNIVERSITY OF MICHIGAN LIBRARIES

Experiment 1

Three soil types were selected (light, medium, and heavy) and planted with young tomato plants in glass jars as shown in figure 1. The moisture percentage in the soils was kept at field capacity for one month in order that the plants might become well established and develop a large root system. At this point the surface of the jar was sealed off with a paraffin-beeswax mixture and the plants allowed to continue growing until they had reached the wilting point. The plants from two jars of each soil type were removed

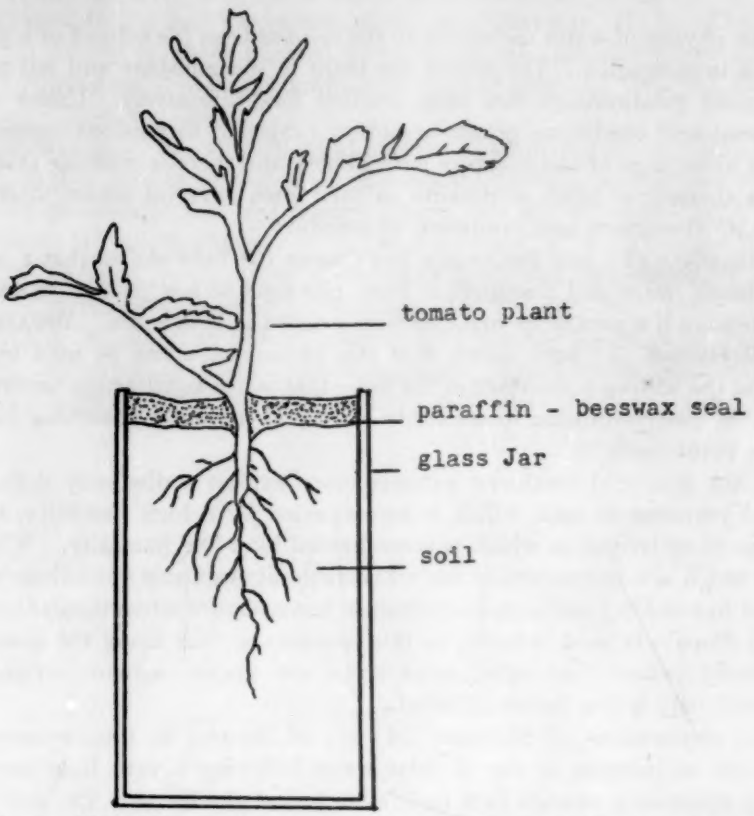

Fig. 1. Method of growing plants in jars of soil protected by a surface seal of paraffin-beeswax mixture.

and the moisture percentage of the soils determined. These data are given in column 2 of table I. At the same time two jars of each soil type, containing wilted plants, were transferred to a saturated atmosphere. Saturation was maintained by using a "fogger" of the type used on vegetable display tables. Two plants of each soil type were removed from the saturated atmosphere at the end of 24 hours and two after 48 hours. The paraffin-beeswax seal and plants were removed and moisture percentage determined in the soil. These data are given in columns 3 and 4 of table I.

The data show a rapid build-up of moisture in the dry soil. This moisture was absorbed by the leaves, transported to the roots, and exuded into the soil. Assuming that field capacity is approximately twice the wilting percentage, the data show that sufficient water was transported through the plants from the foliage to build the soil moisture above the field capacity.

Experiment 2

A similar and confirming experiment was conducted in which eight-week-old tomato plants were transplanted to percolators filled with soil. Sufficient water was added to bring the soil to field capacity and the top of the percolator then sealed off with paraffin-beeswax mixture. The outlet at the bottom of the percolator was left open. These plant cultures were then placed in a fog as in Experiment 1 where they were kept continuously for 12 weeks. In this period of time the plants flowered and set a crop of tomatoes with no other sources of water than that taken up from the fog.

TABLE I

Moisture equivalent of light, medium, and heavy soils; wilting percentage for tomato plants; build-up of moisture in the soils through the leaves

Soil	M. E. of soils	Per cent. moisture at wilting	Per cent. moisture in soils	
			24 hours	48 hours
Light	12.5	6.5	18.5	20.2
Medium	22.7	11.9	25.0	27.0
Heavy	36.8	20.0	39.5	47.2

Experiment 3

In the next experiment moisture absorption through the leaves was studied by a slightly different technic. A series of young tomato plants was sealed in dry, empty 250 ml. Erlenmeyer flasks with glass collars fitted on the stem above the flask as shown in figure 2. The stem was sealed into the cork stopper with paraffin-beeswax mixture. The glass collar was sealed to the cork. The joint between the flask, cork, and collar was then securely taped with rubber tape, thereby forming a water-tight seal. The collar was then filled with air dry soil, a cork was fitted in the top of the collar and sealed securely to prevent entrance of moisture. The plants were then placed in the atmosphere of fog.

After two days the soil in the glass collars was moist and the plants had exuded a small amount of water into the flask. Two weeks later roots were visible in the soil in the glass collar and considerable free water had been exuded into the flasks. At the end of one month the experiment was discontinued and the percentage moisture in the soil from the collar was determined and the volume of water in the flasks measured. The data obtained from two representative plants are given in table II.

UNIVERSITY OF MICHIGAN LIBRARIES

The experiment was repeated with the exception that the Erlenmeyer flask was half filled with tap water. The results were the same—the soil in the collars became saturated and the water volume increased in the flask.

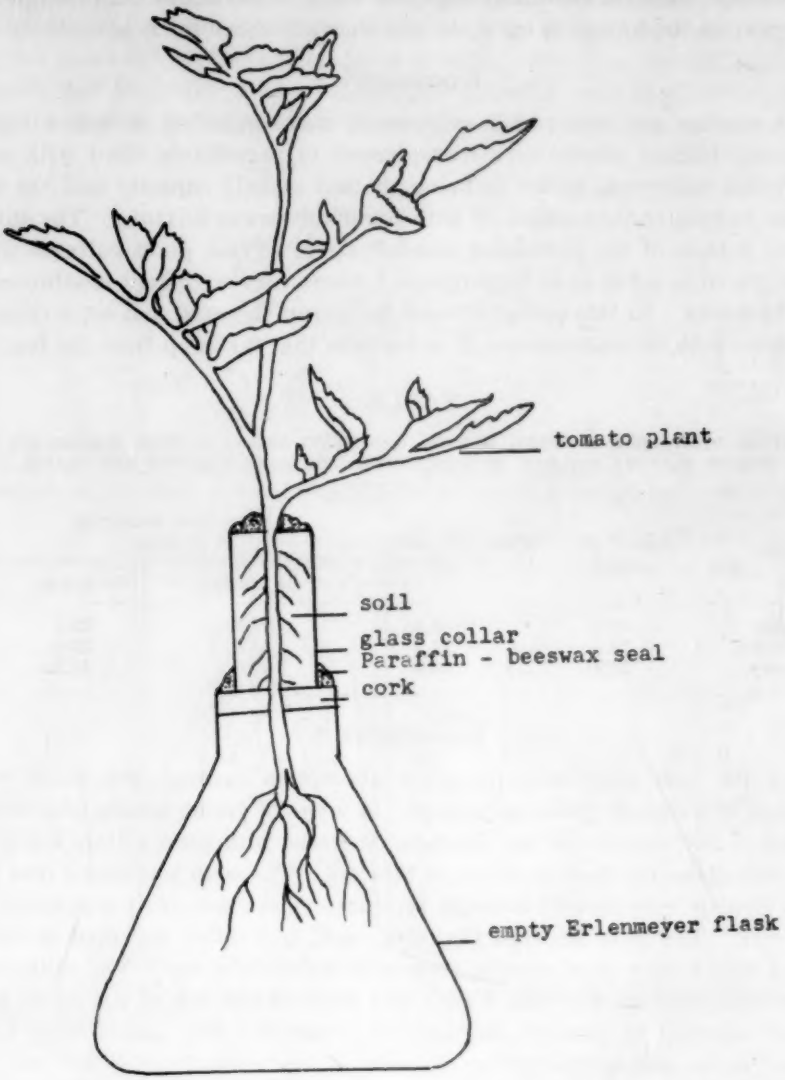

FIG. 2. Method of growing tomato plants with roots in an empty flask with stem collared in soil.

The experiment was repeated several times using soils of different texture in the glass collar. In every case there was a build-up of moisture in the collar to field capacity or higher and free water was exuded into the flasks.

In order to check the security of the paraffin-beeswax seal an experiment was conducted in which the plants were grown in an inverted position. The build-up of water in the soil in the collar and of free water in the empty flask took place at about the same rate as when the plants were grown in an upright position.

TABLE II

TRANSPORT OF ABSORBED MOISTURE FROM LEAVES TO SOIL AND TO EMPTY FLASK

PLANT NO.	ML. WATER IN FLASK	PER CENT. MOISTURE IN SOIL	M. E. OF SOIL	WILTING PERCENTAGE* OF SOIL
1	160	30.1	16.7	9.1
2	185	35.2	16.7	9.1

* CALCULATED FROM MOISTURE EQUIVALENT (M. E.)

At no time during this experiment did the plants show any evidence of a nutritional disturbance indicating that they obtained sufficient nutrients from the soil in the glass collar. Therefore the plant obtained nutrients from the soil even though the soil withdrew water from the plant. The analysis of the free water which had exuded into the flasks indicates that there was a movement of ions from the roots to the moisture film on the root surface.

EXPERIMENT 4

In the last experiment 24 tomato plants were grown in the complete absence of soil—that is, they were sealed in empty 250 ml Erlenmeyer flasks as shown in figure 3. These plants were placed in an atmosphere of fog, as for the previous experiments. Four plants were taken down each week thereafter and the volume of water that had been exuded through the plant into the flasks was measured. These data are given in table IV.

A second series in this experiment was grown under a canopy of cloth in

TABLE III

ANALYSIS OF WATER EXUDED INTO EMPTY FLASK BY TOMATO PLANTS, PARTS PER MILLION

Calcium (Ca)	50	Phosphate (PO_4)	28
Magnesium (Mg)	trace	Nitrate (N)	27
Sodium (Na)	11	Ammonia (NH_4)	none
		Chlorine (Cl)	5

an atmosphere of 100% relative humidity, but one in which a mist of free water did not come in contact with the leaves as in the previous experiments. Here again two plants were taken down each week and the volume of accumulated water in the flasks was measured. These data are given in the last column of table IV.

UNIVERSITY OF MICHIGAN LIBRARIES

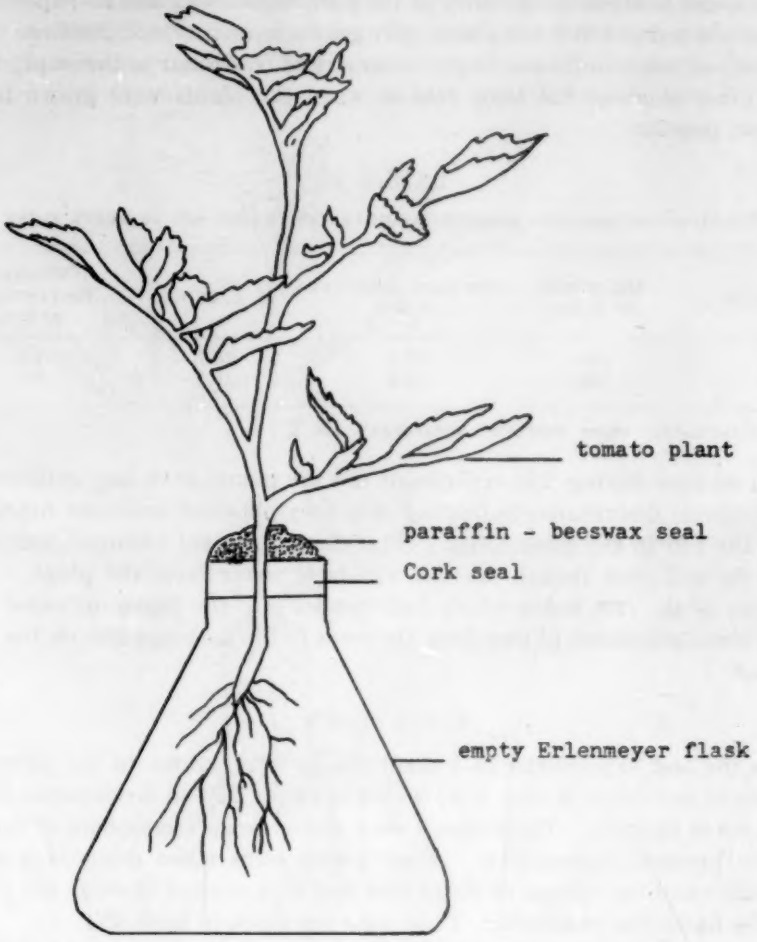

FIG. 3. Method of growing plants in complete absence of soil.

Summary

Under natural conditions the root is the principal water-absorbing organ of plants. These experiments show that when a plant is placed in a saturated atmosphere or fog the leaves become a primary water-absorbing organ. The experiments presented show that in a saturated atmosphere the pressure developed by the absorption of water through the leaves is greater than when water is absorbed through the roots. When a dry soil is "collared" around the stem of a tomato plant growing in a well watered soil the moisture content is built up to the wilting point only (3). In Experiment 3 it is shown that when dry soil is "collared" around the stem of a tomato plant absorbing water through the leaves, from a saturated atmosphere, the build-up of water reaches field capacity or higher.

It was shown in Experiments 3 and 4 that the water absorbed by the plant and exuded by the roots is a considerable quantity. In the exuded water nutrient ions pass out of the roots. It is shown that in a dry soil there may be a withdrawal of water from the roots and of nutrient ions from the soil in an atmosphere of high humidity.

A tomato plant can absorb water from a saturated atmosphere, transport it to the roots, and build up the soil moisture to or above the field capacity.

The absorption is greater and the rate more rapid from a fog which maintains an abundance of free water on the surface of the leaves than from an atmosphere of 100% humidity.

TABLE IV

TRANSPORT OF WATER THROUGH THE LEAVES TO THE FLASK

TIME, WEEKS	VOL. ACCUMULATED WATER, ML.	
	FROM MIST	UNDER CANOPY
1	21.7	8.5
2	50.5	17.2
3	83.3	26.5
4	125.2	.....
5	145.0	.....
6	163.0	.....

Tomato plants will grow to maturity, flower, and set fruit with no other source of water than that absorbed through the leaves from a fog or an atmosphere of 100% humidity.

The flow of water in tomato plants is reversible under saturated atmospheric conditions.

AGRICULTURAL EXPERIMENT STATION
UNIVERSITY OF ARIZONA
TUCSON, ARIZONA

LITERATURE CITED

1. BREAZEALE, J. F. Maintenance of moisture equilibrium and nutrition of plants at and below the wilting percentage. Arizona Agric. Exper. Sta. Tech. Bul. 29. 1930.
2. BREAZEALE, J. F. and CRIDER, F. J. Plant association and survival, and build up of moisture in semi-arid soils. Arizona Agric, Exper. Sta. Tech. Bul. 53. 1934.
3. BREAZEALE, EDWARD and MCGEORGE, W. T. A new method for determining the wilting percentage of soil. Soil Sci. **68**: 371–374. 1949.
4. BRIERLEY, W. G. Absorption of water by the foliage of some common fruit species. Proc. Am. Soc. Hort. Sci. **34**: 277–283. 1934.

UNIVERSITY OF MICHIGAN LIBRARIES

SOME GROSS CORRELATIONS BETWEEN GROWTH ENLARGEMENT AND THE SOLUTE AND WATER RELATIONS OF PLANTS, WITH SPECIAL EMPHASIS ON THE RELATION OF TURGOR PRESSURE TO DISTENTION OF CELLS

T. C. Broyer

(WITH FOUR FIGURES)

Received April 28, 1950

Introduction

In discussions of the dynamics of the growth process, three main views regarding the mechanism of cell enlargement are held. One suggests "that the cell wall must first be subjected to elastic (reversible) or plastic (irreversible) stretching as a result of a turgor pressure developed by the cell sap" (**16**). It is assumed that this is then followed by addition of material substance to the wall through intussusception or apposition, or if unaccompanied by the incorporation of new material, by attenuation of the wall accompanying increase in area. A second idea suggests "that active growth of the cell wall," as a result of "the intercalation of additional molecules between those already present, . . . is the primary step in cell enlargement. Entrance of water into the cell is considered to be a result of the increase in the volume of the cell rather than its cause" (**16**). A third concept, related in part to each of the former, suggests that the initial step is an increased plasticity of the cell wall, followed by a lowering of the turgor pressure and influx of water, tending to approach a new osmotic equilibrium (compare (**12**)). These views, and experimental results in support of each, are presented at length in a recent monograph (**7**). Certain limited experimental data, published earlier (**15**) for other purposes, would appear to afford evidence greatly favoring the first hypothesis. It is desired to represent these data, with further information, and attempt to show that, under the conditions studied, the distention of roots is primarily the result of turgor pressure developed in the internal phases of cells.

Theoretical aspects of turgor in roots

The system to be discussed, involves the root systems of plants in relation to the soil solution under natural conditions. The discussion is based on the simplified, integrated plant osmometer system as treated earlier, elsewhere (**1**). The terminology used, however, is in general that most common in the literature (**7**).

The osmotic equation may be expressed as follows:

$$DPDD\ (NDPD) = (OP_i + MP + nMP_e) - (OP_e + HP'_i + HP''_i + nMP_i).^1 \quad (1)$$

Assuming rapid flux of water across the protoplasmic membrane interposed between the external and internal solution phases, *i.e.*, assuming approach toward osmotic equilibrium, then DPDD = 0.

Under the conditions of the present experimentation any nMP_e is unknown *per se*, but is included, at least in part, in $\triangle T_f$ of soil as measured.[2] Further, nMP_i probably is insignificant in value in the internal phase, since as is assumed here, the internal lumena are probably relatively free of colloidal inclusions. In any event, its value is included, at least in part, in $\triangle T_f$ of sap as measured. Again, since an extrinsic hydrostatic pressure, related to intercellular pressure or tension, or transpiration, is ineffective in the integrated osmotic system here (see footnote 1), then $HP'_i = TP_i$. Finally, the curves of figure 1, especially assuming linearity at their upper OP_e limits, do not suggest the participation of a metabolic pressure in the water relations of this system. Therefore, MP is zero or insignificant. We have for the present discussion then, that

$$TP_i = (OP_i - OP_e). \quad (2)$$

The total work expended by the system with time, in the process of expansion is given by the expression

$$W = \int_{V_b}^{V_a} PdV, \quad (3)$$

in which W is the work expended with change in volume dV in going from Volume V_a to volume V_b, and P is the internal pressure (here the turgor

[1] Symbols and their designation for osmotic quantities of the simplified, integrated osmotic system:

DPDD or NDPD = the diffusion pressure deficit difference or net diffusion pressure deficit.

OP_e = the osmotic pressure of the external bathing medium.

OP_i = the osmotic pressure of the internal medium.

MP = a possible metabolic pressure.

nMP_e = a nonmetabolic pressure in the external phase, for example an imbibitional tension (negative then, in value). See (1).

nMP_i = a non-metabolic pressure in the internal phase, for example an imbibitional tension (negative then, in value). See (1).

TP_i = the turgor pressure within the internal phase of any osmotic system, equal to a resultant internal hydrostatic pressure(HP_i), where $TP_i = HP_i = HP'_i + HP''_i$ (1). TP_i here, is used in the usual sense (3).

HP'_i = the intrinsic hydrostatic pressure in the internal phase (1).

HP''_i = an extrinsic hydrostatic pressure in the internal phase, related, for example, to an intercellular pressure of tension, or tension or pressure arising through transpiration, etc. (1).

[2] Limited information is available (9, pp. 191–192) concerning the influence in solutions of the presence of inert materials possessing large specific surface, on freezing point depressions and concentrations of solute computed therefrom.

UNIVERSITY OF MICHIGAN LIBRARIES

or hydrostatic pressure). At any time t, the work of expansion is given by

$$W = P \triangle V, \quad (4)$$

and will depend in part on the immediate plasticity or elasticity of the limiting surface.

In the above analysis, it was assumed for simplicity of discussion, that approach toward water equilibrium throughout the system was readily attainable. It should be recognized however, that often in nature this is not the case. On the contrary, frequently the rate of water flux is limited by the differential permeability of the protoplasmic membrane (**11**) or other causes. Then, of course, equation 2 is incomplete. The current discussion is based upon the proposition that this equation approximately holds.

Experimental technique and observations

Early methods for measuring the "concentration" of the cell sap of plants included the plasmolytic and the cryoscopic methods. It has been shown (**8**) that certain errors, inherent in the plasmolytic method may lead to aberrant results in osmotic pressure measurements. Under some circumstances, data obtained through the freezing point procedure may also lead to false inferences. In the latter case, the error appears to lie in the pretreatment of the material (**5**). If the plant tissue is pretreated by freezing at low temperatures followed by expression of the composite sap, comparable data at least, may be consistently obtained.

It has been pointed out earlier (**15**) that the osmotic pressure of plant tissue fluids, both from shoots and roots, varies markedly with the time of harvest during the day. This is primarily related to the differential production of organic products of photosynthesis and is modified between tissues by translocation. Another factor which is involved is related to the differential rate of transpiration from the shoots.

The data to be discussed herein were, as far as possible, without objection from the variables referred to. Corn plants were allowed to grow in a silty loam soil in 3-gallon jars. The total soil moisture was maintained throughout the experiment. The concentration of the soil solution was varied by the addition of soluble salts. The same amount of water was added to each jar, and maintained by means of an inverted funnel that extended to six inches from the bottom of the container and a glass tube that penetrated a wax seal at the soil surface. The soil solution was augmented by the addition of different amounts of a solution consisting of a Shive 3-salt mixture *viz.*, calcium nitrate, magnesium sulphate and potassium phosphate. The plants were placed into a dark chamber for one day preceding experimental study. This treatment minimized the effect of organic solutes in the tissues as well as the transpiration factor. The roots were carefully separated from the soil mass and washed with distilled water. This treatment, though brief in order not to modify

significantly the osmotic pressure value of the roots, tended to further minimize the transpirational factor therein. These conditions, therefore, yielded root material with which equation 2 would probably hold fairly closely. The roots were frozen in closed containers, followed by thawing

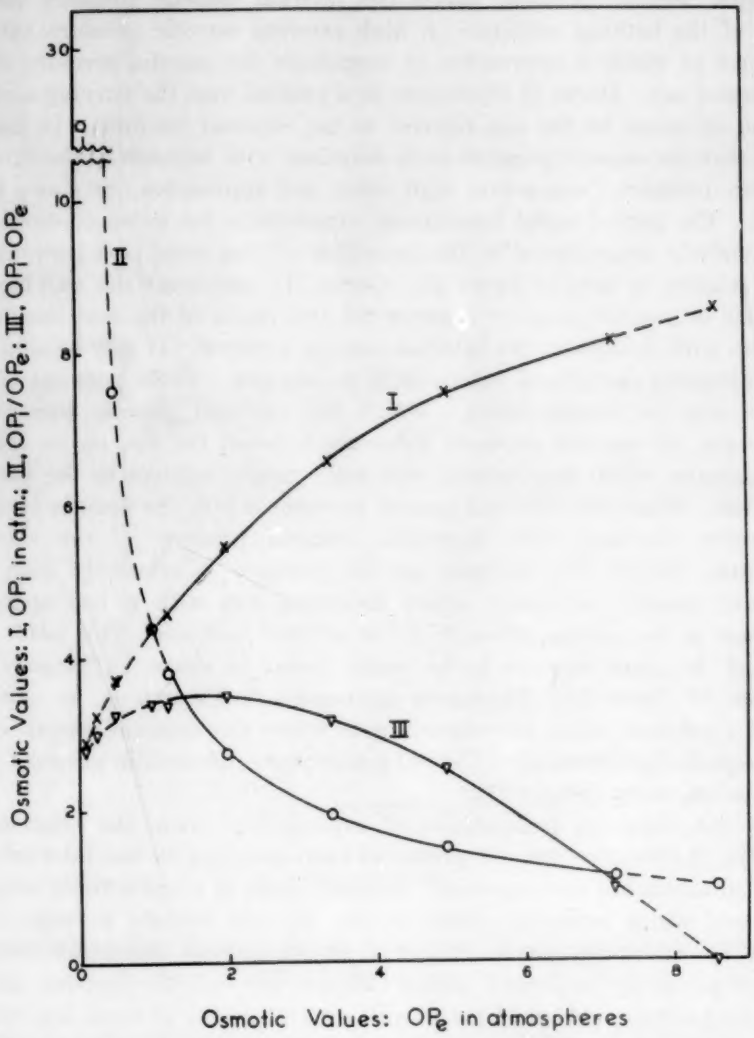

FIG. 1. Relationships between the osmotic pressures of internal and external media of roots.

and expression of composite sap at a pressure of 300 kg. per square centimeter. Freezing point depression measurements were made on this expressed sap as well as on the relatively undisturbed soil mass. The data obtained are shown in table I. From the observed data, osmotic pressures have been calculated and other values computed as indicated elsewhere.

The osmotic pressure relations between root sap and soil solution are shown in figure 1. Several conditions may be observed. Curve I represents the relative osmotic pressures of the internal and external media of the root osmometer system. It may be seen, that over a wide range of external osmotic pressure values the internal osmotic pressure exceeds that of the bathing medium. A high external osmotic pressure value is reached at which it approaches in magnitude the osmotic pressure of the expressed sap. Curve II represents, in a general way, the varying accumulation of solute in the sap relative to the external medium. It may be seen that the osmotic pressure ratio decreases with increase of the external osmotic pressure, from a very high value, and approaches unity as a lower limit. The partial molal free energy expenditure for accumulation is approximately proportional to the logarithm of this ratio (see later discussion relative to data of figure 2). Curve III represents the varying difference in osmotic pressure between the two media of the root osmometer system with changes in the external osmotic pressure. It may be seen that a complicated curvilinear relationship is obtained. Three portions of this curve may be distinguished. Where the external osmotic pressure is moderate, the osmotic pressure difference between the two media reaches a maximum which may extend over some range, relative to the bathing solution. Where the external osmotic pressure is low, the osmotic pressure difference decreases with decreasing osmotic pressure of the external medium. Where the external osmotic pressure is relatively high, the osmotic pressure difference again decreases, but with a corresponding increase in the osmotic pressure of the external medium. This latter portion of the curve appears to be nearly linear in shape. (Compare discussion of Curve I.) The curve approaches the x axis at an external osmotic pressure value corresponding to where the internal osmotic pressure equals that externally (Curve I) and where the osmotic pressure ratio approaches unity (Curve II).

In the foregoing presentation of experimental data, the relationship between the relative osmotic pressures corresponding to the internal and external media has been stressed. Another phase of plant activity must be discussed which primarily bears on the internal osmotic pressure level attainable under any particular set of circumstances. Assuming that the solutes primarily concerned within the root for osmotic purposes are of inorganic nature, obviously the overall concentrations of these are important. Under the conditions discussed, internal concentrations are higher than external concentrations, throughout. From earlier work (**2**) it is clear that diffusion of solutes is not primarily concerned in the net accumulation of inorganic solute required. Although various modes of influx are involved, the primary means of influx is related to the net inward movement associated with metabolic energy expenditures. From the internal and external osmotic pressure values, an approximation of the

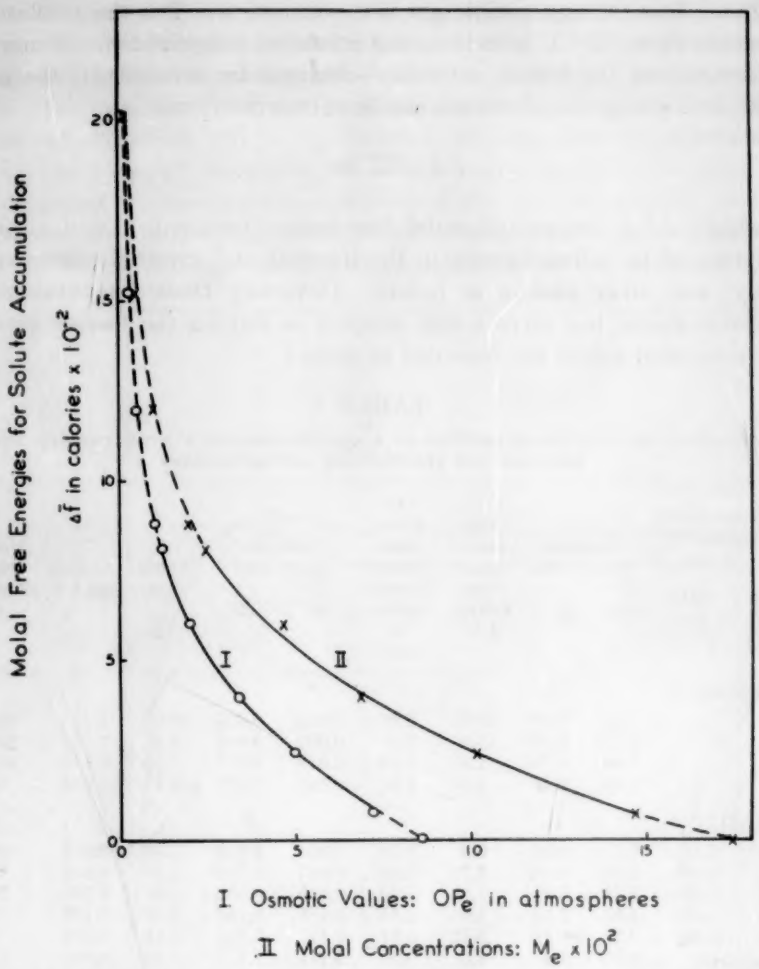

FIG. 2. Relationships between the free energies for inorganic solute accumulation in roots and the salt level in the bathing medium.

corresponding salt concentrations can be calculated and from these, the energy expenditure, per mole of salt accumulated, on the part of the plant roots.

Computation of the energy requirement per mole of inorganic solute accumulated, at any particular set of relative values of the internal and external media, has been made in the following manner (**2**). The concentrations[2] of the two solution phases have been calculated from

$$OP = i\ CRT, \qquad (5)$$

in which i is the van't Hoff coefficient, C is the solute concentration in moles per liter (which may be corrected to molalities from density infor-

mation), R is the appropriate gas law constant, and T is the absolute temperature (here, 25° C is used as the reference temperature). From these concentrations (or better, activities—obtained by correction), the partial molal free energy requirements can be calculated from

$$\Delta\bar{f} = RT \ln \frac{a_i}{a_e} \qquad (6)$$

in which, $\Delta\bar{f}$ is the partial molal free energy required, a_i and a_e are the activities of the solute species in the internal and external phases respectively, and other factors as before. Obviously these computations are approximations, but serve a real purpose in linking the factors involved. The computed values are recorded in table I.

TABLE I

RELATIONS OF THE OSMOTIC QUANTITIES OF A ROOT OSMOMETER SYSTEM AND THE ENERGIES REQUIRED FOR ELECTROLYTE ACCUMULATIONS

FREEZING POINT DEPRESSIONS		OSMOTIC PRESSURES		OSMOTIC PRESSURE RATIOS	OSMOTIC PRESSURE DIFFERENCES	CONCENTRATIONS		CONCN. RATIOS	MOLAL FREE ENERGIES
SOIL ΔT_f (° C)	SAP ΔT_f (° C)	e ATM.	i ATM.	i/e	i-e ATM.	e M	i M	i/e	log i/e	CAL.
(Computed)*										
		0.10	2.90	29.0	2.8	0.002	0.059	29.6	1.471	2010
		0.25	3.25	13.0	3.0	0.005	0.067	13.1	1.114	1520
		0.50	3.75	7.5	3.25	0.010	0.077	7.51	0.876	1195
		1.00	4.40	4.4	3.4	0.020	0.090	4.41	0.644	880
(Observed)										
0.101†	0.381†	1.21	4.59	3.8	3.38	0.025	0.094	3.90	0.591	807
0.165	0.455	1.99	2.48	2.75	3.49	0.047	0.112	2.75	0.439	559
0.281	0.549	3.38	6.61	1.96	3.23	0.069	0.135	1.96	0.291	397
0.412	0.624	4.96	7.51	1.51	2.55	0.102	0.153	1.50	0.176	240
0.600	0.680	7.22	8.19	1.13	0.97	0.147	0.167	1.14	0.054	74
(Computed)		8.60	8.60	1.0	0.00	0.175	0.175	1.00	0.000	0

* Values computed from extrapolations of curve I, figure 1.
† Data of MCCOOL and MILLAR (15).

The partial molal energy expenditure requirements are plotted in figure 2. The energy quantities are related here to both the varying external osmotic pressure as well as the concentration for reference in discussion. It may be observed, that at low values of external osmotic pressure or concentration a very high energy per mole of salt influx is required, decreasing rapidly with increase of the external values of concentration. At high external osmotic pressures or concentrations, little energy expenditure is required per mole of salt accumulated, approaching zero where the relative concentrations of the solutions in the two phases approach each other. Energy here may be expended through a salt respiration, but thermodynamically little to no energy is required except possibly in main-

tenance of the relative solute levels in the dynamic efflux and reinflux of salt at an established steady state for specific ion species characteristically associated with the plant type.

The effectiveness of root respiration for solute accumulation has been pointed out elsewhere (**17**). In figure 3, the approximate relationship between the rates of accumulation of salt and respiration in cells are plotted against the varying concentration of the external medium. Curve I shows that the solute accumulation rate increases with increase of the concentration of the external medium approaching a limiting maximum rate

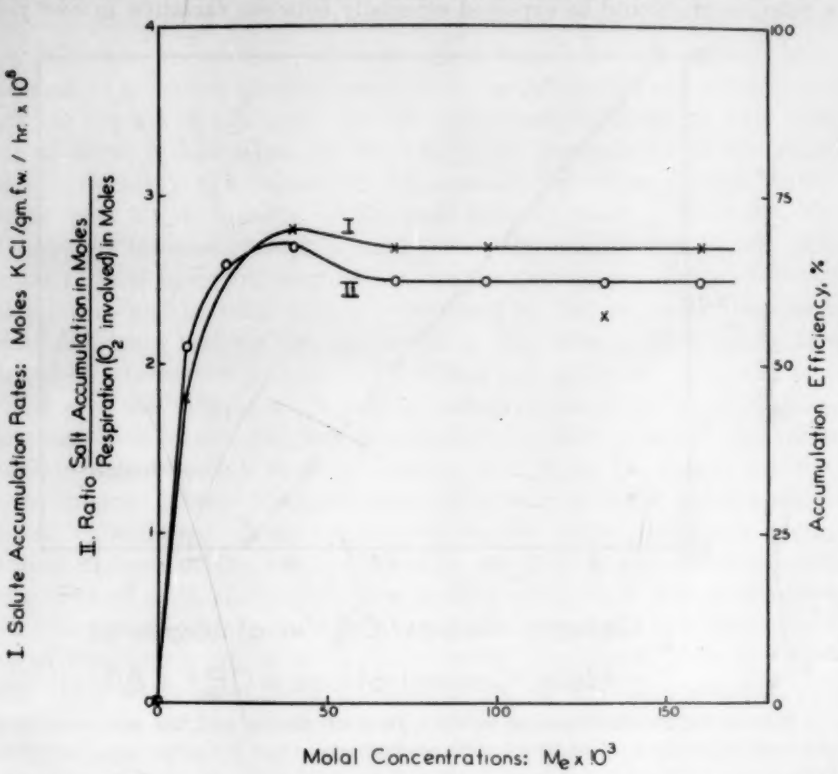

FIG. 3. **Relationships among the rate of inorganic solute accumulation in roots, the efficiency of oxidative metabolism for accumulation, and the salt level in the bathing medium. Reproduced with modification, from ROBERTSON and WILKINS (16).**

at from 20–40 millimoles per liter of external salt, for storage tissues of carrot. The maximum was attained with barley roots at somewhat lower levels (**10**). Curve II, where the solute accumulation per unit respiration increase is plotted against the concentration of the external medium, shows a trend similar in form to that of curve I and nearly congruent. The important suggestion which emerges is that the efficiency of the respiratory energy supply for accumulation, approaches a maximum level at about 20–40 millimoles per liter of external salt supply. This is more

evident when plotted as a percentage efficiency (see curve II when expressed in terms of per cent. efficiency on the right hand ordinate based on the thesis of electron transfer in accumulation of solute through an associated oxidation-reduction process (**17**)). It should be particularly observed, that the accumulation efficiency of salt respiration decreases rapidly from this maximum as the concentration of the external solution decreases.

One further line of experimental evidence may be referred to at this point. From the nature of the turgor pressure curve (curve III, fig. 1), a relationship would be expected especially between variation in root yield

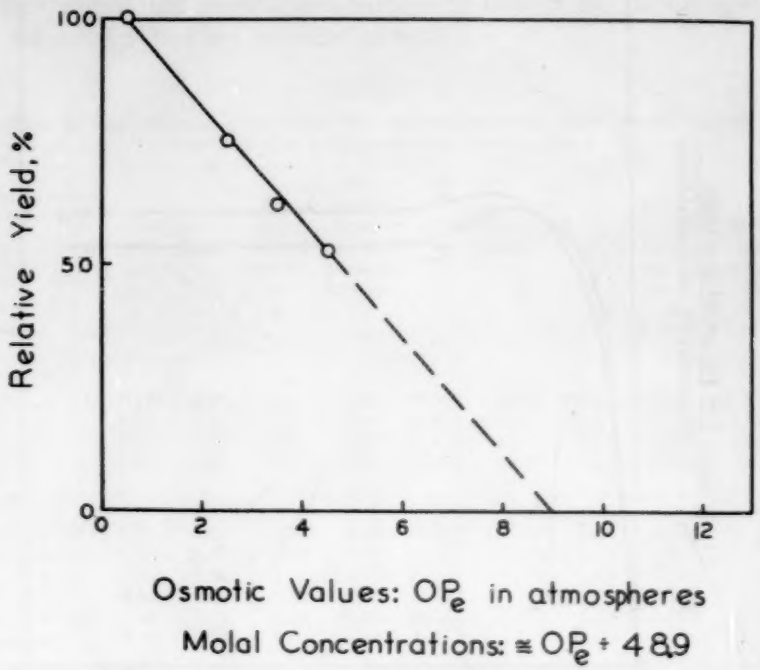

FIG. 4. Relationship between relative yield of alfalfa and the salt level in the root bathing medium. Reproduced with modification, from MAGISTAD and REITEMEIER (**11**).

and variation in osmotic pressure of the external solution. It has been shown earlier, in studies of plant yield under saline conditions, that over a fairly wide range of external solute concentrations, there is a linear negative correlation between the relative growth of the plant and the salt level bathing the roots. This is exemplified by figure 4. Like observations have been obtained for diverse types of crops (**13, 14**), other conditions being similar. The only variation from that shown in this graph is that "Salt-tolerant crops have a slightly sloping regression line, whereas salt-sensitive crops have a steeply sloping one" (**13**). The similarity of this line (fig. 4) with that of the osmotic pressure difference between the in-

ternal and external media in figure 1, particularly where higher external solution concentrations are concerned, should be noted. Compare beet yields in figure 2 (**14**) and discussion thereof on page 155, therein.

Discussion

The various lines of experimental data would appear to point toward certain gross correlations between growth and the solute and water relations of plants. An important sequence of processes seems to be involved at any particular time, especially during the grand period of growth. The first step seems to be concerned with the presence of an increased respiration (oxidative metabolism) of roots where bathed by salt media. Inorganic solute is accumulated in accord with regulatory influences associated with the process of salt influx for the particular plant species (**4**). The rate of influx is determined by the differential permeability of the protoplasm to the solute as accumulated, the concentration of the solution (modified in part by the quality of the ionic supply) bathing the roots, the efficiency of the energy supply, and the energy requirement at any particular ratio of inorganic solute concentration (or better activity) between the internal and external phases. Governed by the particular inorganic solute difference between the two media at any time t, water moves into (or out of) the system by osmosis. The rate and extent are primarily concerned with this difference. Where and when extrinsic factors (transpiration, imbibition by soil, etc.) are not dominant in their influence, this solute concentration difference in large measure determines the turgor pressure in the internal phase. Turgor distention of cells on water entry leads to over-all enlargement of the organ with further surface exposure to the bathing medium. Attended by favorable cell division and differentiation, the growth of roots, and possibly less directly of shoots, is thus determined. "In other words, enlargement of plant tissues—growth—is a direct function of turgescence which in turn is partially conditioned upon the force with which water is withheld from the plant. It has been observed . . . that there is a fairly close relationship between vegetative growth and the average moisture stress, if other factors affecting growth are not limiting and are kept as uniform as possible" (**18**).

Although several factors enter into the sequence of growth, it would appear that turgor pressure holds a key position therein. (Compare especially curve III, figure 1 with figures 2, 3 and 4). Thus, where turgor pressure (see equation 2) approaches zero, distention of roots should not continue. At low turgor pressures, growth is restricted. Thus, on the one hand the distention may be limited at too low an external solute concentration where permeability and effective energy supply are adverse. Again, the enlargement may be restricted at too high an external solute concentration due to a limited attainable osmotic pressure difference, even though the efficiency of the energy supply for solute accumulation may be

more favorable. There appears to be an optimum, intermediate range of external solute concentrations where all factors operate in mutual accord toward a maximal turgor pressure within cells, and maximal growth.

One of the most intensive and extensive studies on growth has been carried forward with sugar cane in Hawaii. Here, plant enlargement has been particularly related to the water regime applied to the crop (**6**). It would seem that here as well, the prime factor concerned is the turgor pressure maintenance during development.

Although the discussion herein has revolved around the root and its environment, this relationship of turgor to growth enlargement is probably extendable to include the shoot and fruiting bodies (compare earlier quotations from (**18**)). Here however, other factors may be of more importance relative to turgor pressure, or circumstances may be such that less favorable correlations may be observed with other organs. However, the growth data obtained under various conditions of salinity around roots suggest that the enlargement of the plant as a whole is related to turgor pressure in a large degree.

It should be recognized, that there may be times in the production of crops when maximum enlargement growth is not desirable for one reason or another (cf. (**6**)). This for example might be the case during the late fall with winter cereals or in later stages of the development of crops where maturation of the fruiting bodies or storage organs requires relatively less enlargement in favor of the storing up of compounds of commercial and/or nutritional value.

One should not infer that other factors than turgor pressure do not optimum concentration (in the external medium, for best growth) should directly or indirectly come into play to modify growth enlargement. Limitation in the supply of any growth factor will certainly, and often critically, restrict the development of plants in accordance with Mitscherlich's law. Certain compounds supplied from without or produced within plants may prove noxious to growth. On the other hand, growth hormone studies indicate that compounds may be formed within plants which at favorable concentrations may allow more optimal growth enlargement. Also, it has been shown that water deficits may modify other process rates, *e.g.*, photosynthesis and respiration (**11**).

The main thesis of this discussion is clear: "It would appear that the furnish an ample supply (rather, should afford conditions for maximum relative accumulation) of nutrients, yet have such a low osmotic value that water absorption is not markedly reduced." (other conditions being favorable).[3] The particular values in any of the curves, their shapes etc., are probably subject to variation depending upon the species of plant and environmental conditions. Experimentation now in progress should yield important information on variations from the general trends herein

[3] Parentheses here are author's inserts in quotation from (**14**) page 163.

described. It may be suggested, that it can be envisioned that as physiologists now diagnose deficiency incipience for inorganic elements by foliar analysis, so a progressive analysis of the freezing point depressions of soils and the root saps of crops could yield information in large scale plantings which could afford important information on the future regime of water-solute supply for optimal crop production.[4]

Conclusions

An analysis of the sequence of factors concerned in the growth enlargement of cells is presented. It is inferred from the data presented, that turgor pressure or attendant conditions within cells is the primary factor concerned with their distention or growth enlargement in association with the laying down of new cells through division and differentiation. Even the latter may be modified by the former. In that a maximal turgor pressure is desirable under most circumstances, the inorganic solute ratios and total concentrations in the external medium relative to that in the plant are an immediate concern of the physiologist. It is suggested that progressive information on the turgor pressure within roots as a crop is developing, may be of diagnostic value to the agriculturalist in order to obtain maximum yield of crops.

DIVISION OF PLANT NUTRITION
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BROYER, T. C. The movement of materials into plants. Part I. Osmosis and the movement of water into plants. Bot. Rev. **13**: 1–58. 1947.
2. BROYER, T. C. The movement of materials into plants. Part II. The nature of solute movement into plants. Bot. Rev. **13**: 125–167. 1947.
3. BROYER, T. C. On the theoretical interpretation of turgor pressure. Plant Physiol. **25**: 135–139. 1950.
4. BROYER, T. C. The nature of the process of inorganic solute accumulation in roots. In Symposium on Mineral Nutrition of Plants. University of Wisconsin Press. Madison, Wisconsin 1950.
5. BROYER, T. C. and HOAGLAND, D. R. Methods of sap expression from plant tissues with special reference to studies on salt accumulation by excised barley roots. Amer. Jour. Bot. **27**: 501–511. 1940.
6. CLEMENTS, H. F. Environmental influences on the growth and mineral nutrition requirements of sugar cane. In Monograph on Mineral Nutrition of Plants. Univ. of Wisconsin Press. Madison, Wisconsin 1950.

[4] Similar information may be possible by measurements with soil tensiometers after correlation of tensiometric data with information of the present sort.

7. Crafts, A. S., Currier, H. B. and Stocking, C. R. Water in the Physiology of Plants. Chronica Botanica Co., Waltham, Massachusetts 1949.
8. Currier, H. B. Water relations of root cells of *Beta vulgaris*. Amer. Jour. Bot. **31**: 378–387. 1944.
9. Gortner, R. A. Outlines of Biochemistry. J. Wiley and Sons, Inc. New York. 1938.
10. Hoagland, D. R. Salt accumulation by plant cells, with special reference to metabolism and experiments on barley roots. Cold Spring Harbor Symposia on Quant. Biol. **8**: 181–194. 1940.
11. Kramer, P. J. Plant and Soil Water Relationships. McGraw-Hill Book Co. New York 1949.
12. Levitt, J. The role of active water absorption in auxin-induced water uptake by aerated potato discs. Plant Physiol. **23**: 505–515. 1948.
13. Magisitad, O. C. and Reitemeier, R. F. Soil solution concentrations at the wilting point and their correlation with plant growth. Soil Sci. **55**: 351–360. 1943.
14. Magistad, O. C., Ayers, A. D., Wadleigh, C. H., and Gauch, H. G. Effect of salt concentration, kind of salt, and climate on growth in sand cultures. Plant Physiol. **18**: 151–166. 1943.
15. McCool, M. M. and Millar, C. E. The water content of the soil and the composition and concentration of the soil solution as indicated by the freezing point lowerings of the roots and tops of plants. Soil Sci. **3**: 113–138. 1917.
16. Meyer, B. S. and Anderson, D. B. Plant Physiology. D. Van Nostrand Co. New York 1939.
17. Robertson, R. N. and Wilkins, M. J. Studies in the metabolism of plant cells. Australian J. Sci. Res. Series B. **1**: 17–37. 1948.
18. Wadleigh, C. H. and Gauch, H. G. Rate of leaf elongation as affected by the intensity of the total soil moisture stress. Plant Physiol. **23**: 485–495. 1948.

IS THE AMERICAN ELM (*ULMUS AMERICANA*) INJURED BY NATURAL GAS?[1]

FELIX G. GUSTAFSON

(WITH ONE FIGURE)

Received October 6, 1949

Several years ago the writer (1) reported some investigations on the influence of natural gas on flowering plants. This report stated that 12 species of plants exposed to 1% natural gas in air from one to three weeks suffered no injury. Bougainvillea and Swainsonia were injured by a 2% concentration over a period of 14 days. Five species of seeds germinated as well in 1.5 to 5% natural gas in air as they did in an air stream.

As a result of the findings that most flowering plants and seedlings were not injured by low concentrations of natural gas, it became desirable to set up experiments with trees. Popular reports that trees are injured by escaping manufactured gas are quite common. Does the natural gas percolating through the soil from broken mains do the same?

Methods and results

A preliminary experiment with Chinese elms was run during the summer of 1943. Three lots of two similar trees of about four to five feet tall were cut back to about 30 inches and planted in porous soil in wooden tubs 13 by 15 inches.

Figure 1 illustrates the arrangement of the gas-air distributing pipe and the drainage system as arranged in the tubs. Before planting the trees, enough coarse gravel was placed in the bottom of the tubs to just cover the pipe B and the opening A was plugged with a cork, which remained in place, except at times of watering when it was removed to allow excess water to drain off. The initial gas concentration as it entered the tubs was 1.5% and attempts were made to maintain this concentration during the summer, but there was some variation. The gas concentration in the soil was measured now and then during the summer and it was never found to be over 0.5%. One tree from each pair had the gas-air mixture pass through the soil in which it grew and the other tree of each pair had air passing through the soil. The tubs with the trees were stationed on the southwest side of a small greenhouse on the campus, where direct sunlight reached them during part of the morning and all afternoon. In the fall the tree tops and roots were examined for injury. As far as could be detected the gas had not injured the trees in any manner.

In October 1943 six American elm trees, between five and six feet tall, were selected as three pairs, which were carefully matched. All trees were

[1] Paper from the Department of Botany of the University of Michigan, No. 896, in cooperation with the Michigan Consolidated Gas Company, Detroit, Michigan.

pruned to about five feet and planted in a rich porous soil in the above used tubs. The experimental trees were numbered 11, 13 and 15, and the controls 12, 14 and 16. The tubs and trees were lowered in a cold frame, at the Botanic Garden, where they were stored each successive winter. Later in the fall, leaves were packed between the tubs, but no leaves were put on top of the soil.

SEASON OF 1944.—The trees wintered very well and were brought to the campus early in May and put in the same place the Chinese elms had oc-

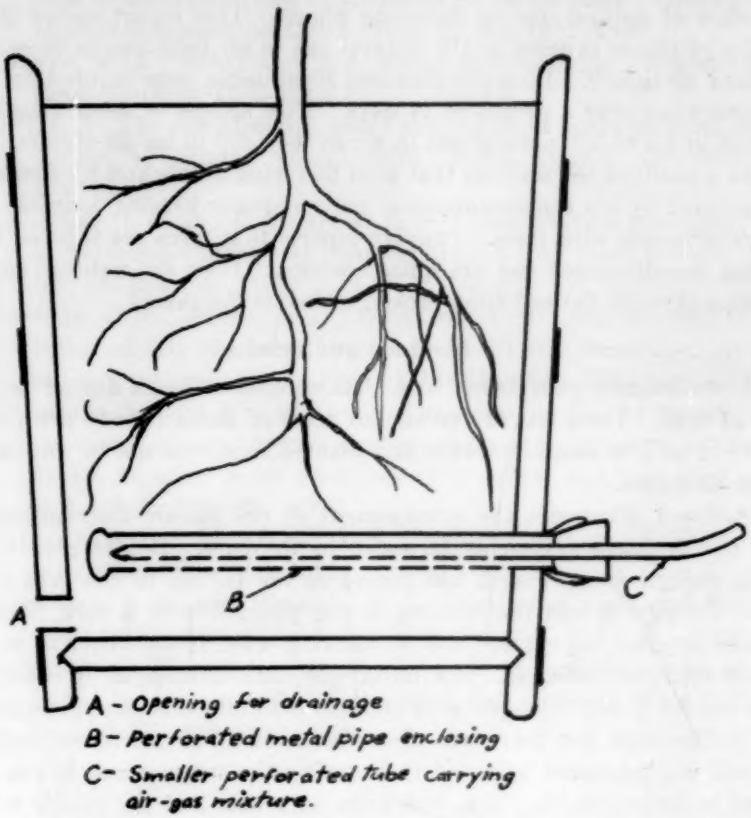

FIG. 1. A longitudinal section of the tub in which the trees were grown, showing the arrangements of the gas-air and air inlet pipe.

cupied the previous season. On June 1 the leaves and branches were well developed and had nearly reached their full growth. Tubs 11, 13 and 15 were connected to the gas-air mixture line and the controls 12, 14 and 16 to the air line. The gas concentration was set at 3.0% and an attempt was made to keep it at that during the summer but frequently it became higher, though never lower. Frequent tests of the soil gave gas concentrations ranging from zero to 1.6%.

On November 6, the root systems were examined by removing the trees from their tubs, but this was a little difficult as the soil was partly frozen. The pair, 11 and 12, had many large roots, but control tree, 12, had more small roots; the root systems of 13 and 14 were essentially the same; the pair, 15 and 16, had an abundance of roots, but as in 12, the control 16 had more fine roots. The soil in tubs 11 and 15 was frozen more than in the other tubs and in removing the roots from the soil, more of the fine roots may have broken off. There was some indication of this in the frozen chunks of soil. None of the roots showed any sign of injury. The trees were replanted in fresh porous soil and wintered as during the preceding season.

Season of 1945.—On June 9, the trees seemed to have reached their full growth and the tubs were connected to their respective gas-air or air lines. The concentration of gas was fixed at 3.5%. This season the gas concentration fluctuated considerably, sometimes decreasing and other times increasing over 3.5%. The concentration was, however, daily adjusted to 3.5%. The gas concentration in the soil was frequently determined. This also fluctuated a great deal. The concentration ranged from zero to 2.7%, but most of the measurements showed a gas concentration around 1%.

Two weeks after the experiment was started for the season, the heights of the trees were measured. These measurements were: tree 11, 70 in.; 12, 73 in.; 13, 66 in.; 14, 65 in.; 15, 68 in.; and 16, 65 in. The odd numbers denote the experimental trees.

In order to avoid freezing of the soil as happened the previous year, the trees were removed from the soil on September 27. The roots of the trees growing in soil receiving the gas-air mixture were darker and there were fewer fine roots than on trees growing in soil receiving only air. Later, microscopic examination of the fine roots brought out the fact that the dark roots were injured and the bark separated readily from the wood, whereas in the lighter brown roots, the bark could not be separated from the wood. The dark rootlets were probably dead.

Later in the fall, the total growth in length for the season was determined by measuring the individual twigs. This growth had been made from buds formed during the preceding summer when the roots of the plants were exposed to gas. Table I gives the results. It will be noticed that the total growth as well as the average per branch was higher for the gas in air-treated plants than for the controls. In the former, the average growth per branch was 12.7 cm and in the latter 9.3 cm. per branch.

Season of 1946.—In the fall of 1945 it had been decided to discontinue the experiment and the trees were planted in the field. However, the unsatisfactory condition of the roots, which was disclosed by later microscopic examination, made it desirable to continue the experiment. Early in April, before the buds had opened, the trees were dug up and planted in larger

tubs (20 in. deep and 21 in. in diameter). The buds opened normally and by June 26, when gas-air mixture and air was connected to the tubs, the trees were all in good condition and were none the worse for having been dug up an extra time. The gas concentration was set for 3.5% but as in previous summers there was considerable fluctuation. The gas concentration in the soil was checked nearly daily and for the greater part of the summer it was above 1%, but the range was from zero to 2.9%.

In October the trees were removed from the tubs and the soil carefully washed from the roots. As in the fall of 1945, the roots of the experimental trees were in parts quite dark, but many regions were light brown with numerous fibrous roots. The control trees also had some dark areas on the root system, but they were less extensive than on the experimental trees. The root systems of the experimental trees were somewhat less dense than those of the control trees. Before replanting, the roots were severely pruned on all trees.

TABLE I

TOTAL GROWTH IN LENGTH MADE DURING THE GROWING YEAR OF 1945

NATURAL GAS IN AIR				AIR			
TREE	TOTAL GROWTH IN CM.	AVE. PER BRANCH	NO. OF BRANCHES	TREE	TOTAL GROWTH IN CM.	AVE. PER BRANCH	NO. OF BRANCHES
11	585.3	10.8	54	12	543.1	8.7	62
13	781.2	14.7	53	14	665.9	12.3	54
15	732.2	12.7	58	16	620.4	7.7	80

SEASON OF 1947.—This season the gas concentration varied from 3.0 to 4.0%. During the early part of the season the gas distribution in the soil was quite uniform as indicated by the bubbling, when the tubs were flooded with water, but toward the end, most of the bubbling was near the periphery of the containers, but this was probably not a disturbing factor as most of the roots were also located in this region. The gas concentration in the soil ranged from zero to 3.3%.

Observations of the root systems in the fall of 1945 and 1946 had shown that the experimental plants had somewhat fewer fibrous roots and also a larger number of roots were dark in color, indicating injury, than on the control trees. Under the conditions of the experiment this had not affected the top growth, but what would happen if the plants were grown under drought conditions? Could the roots absorb enough water to maintain the turgidity of the leaves? To obtain an answer to this question, the soils were saturated with water on September 9 and the tubs covered with cones of tar paper to prevent addition of water by rain or excessive evaporation. The condition of the leaves was observed at intervals and the soil moisture determined in the several tubs. The results were inconclusive.

One reason may have been that the leaves were too mature to show easily recognizable symptoms of wilting. Yellowing and defoliation was also observed and attempts made to correlate this with the treatment of the trees and soil moisture, but to no avail.

At the time the soils were covered with tar paper cones the diameters of the trees were measured a foot above the soil. Table II gives these data.

The greater diameter of the control trees is the first indication found that the somewhat poorer root system of the experimental plants may cause a decreased growth of the tops. This is, however, not very strong evidence, as the original diameters were not known, and the 1948 measurement shows that the experimental trees grew more in diameter than the control trees.

When the roots were examined in the fall, after careful removal of the soil with a stream of water, it was found that they were all very good. The

TABLE II

DIAMETER OF THE TREES ONE FOOT ABOVE THE GROUND AS MEASURED SEPTEMBER 9, 1947, AND SEPTEMBER 10, 1948

TREES RECEIVING GAS				TREES RECEIVING AIR			
TREE	DIAM. IN CM. 1947	DIAM. IN CM. 1948	% INCREASE IN 1948	TREE	DIAM. IN CM. 1947	DIAM. IN CM. 1948	% INCREASE IN 1948
11	2.00	2.26	13.0	12	2.40	2.56	6.7
13	1.83	2.30	25.5	14	2.15	2.50	16.3
15	2.03	2.18	7.4	16	2.38	2.46	3.4
Average increase in 1948		15.3%					8.8%

dark areas were fewer than they were the preceding fall. For the purpose of photographing the different root systems, they were held up against a light background and this brought out the fact that the experimental trees had thinner root systems than their controls.

SEASON OF 1948.—This season the gas treatment began June 4 and ended October 5. The gas concentration varied as usual somewhat, but attempts were made to maintain it between 3.0 and 4.0%; adjustments were made daily. Throughout the summer, the gas distribution in the soil was not very uniform. Tubs 11 and 15 gave concentrations less than 1%, yet tests showed that there were no leaks in the tubs and that the inlet concentration was the same as that passed into tub 13, where concentration was usually around 2.0%.

On August 16 the soil was thoroughly saturated in all the tubs and the tubs covered with tar paper cones as in the preceding summer. No observations were made until September 1, when the following notes were taken:

Tree 11—All leaves on, but dried.

Tree 12—Leaves green and expanded.

Tree 13—Leaves green, but edges rolled a little.

Tree 14—Some leaves dried; others still green.

Tree 15—Leaves green and expanded, but not quite so good as in 12.

Tree 16—Leaves green and not rolled, about as in 15.

September 25 supplied a further observation on leaf condition:

Tree 11—Leaves completely dried.

Tree 12—About 50% still green and some expanded.

Tree 13—Green leaves fewer than on 12.

Tree 14—Most of the leaves have dropped, but a few (less than on 13) are green.

Tree 15—Only apical leaves are green (about as on 14).

Tree 16—About the same as 14 and 15.

The above data shows that the roots of the gas-treated trees were able to supply the tops with sufficient water even though the soil moisture content was not very high.

The diameters were remeasured in September. The data is recorded in table II alongside of the 1947 data. Every experimental tree grew more in diameter than its control and the average for the three experimental trees is 13.3 as compared with 8.8% for the control trees. The diameters of the experimental trees are still less than that of the control trees, but they seem to have gained during this season.

The final examination of the root systems was made on October 5. The root system of plant 11 was quite good and only slightly poorer than that of 12; many of the roots of tree 13 were dark and as a whole the roots were superficial, while the roots of tree 14 were brown, and uniformly distributed through the soil; tree 15 had many dark roots, whereas 16 had only a few dark ones.

Discussion

When the experiment was set up in 1943, it was realized that as the soil froze, it would be impossible to pass gas through it, so no attempt was made to conduct the experiment during the winter. Then the decision had to be made as to how early in the spring the gas should be introduced, and it was finally decided to wait until the leaves had unfolded and nearly reached their full growth; this stage would be easy to determine each season. There was no reason to expect that the gas treatment of the roots would influence the unfolding of the leaves, as that process depends on the water absorbed by the roots and in the spring the soil is always well supplied with water.

KRAMER (**2**) has shown that even dead roots absorb water if the plant is transpiring, i.e., if there is an upward movement of water in the stem, producing a diffusion pressure deficit in the roots. The problem was then thought of as an exposure of the root system to the gas during the real growing period of the year, when there was synthesis of foods and the

formation of cells in the cambium, roots and buds; and during a period when the water is not always so abundant in the soil as it is in the spring.

The distribution of the gas in the soil was not uniform, which would have been impossible, but much of the time bubbles of gas were seen to rise in all parts of the surface when the tubs were flooded, showing at least, that there were channels for air escape distributed in all parts of the soil. However, toward the end of each season these channels became fewer and sometimes the air or gas escaped only near the periphery of the container. All of this probably accounts for the spotty appearance of the root system, where only part of the root system was black, and another light brown. It is very likely that parts of the soil received the full concentration of the gas as it was introduced, whereas other parts received a much lower concentration.

The earlier experiments of the author showed that such low concentration of gas, as might escape from the soil in which the trees grew, into the atmosphere would have no effect on the above ground part; and the effect on that part would be only indirect. If the gas injured the roots they might absorb less water and minerals than uninjured roots.

There has been an injurious effect of the gas on the roots. The root systems that grew in soil receiving gas-air mixture were in some instances smaller than those receiving air, and sometimes there were more dead roots in the former than in the latter, though it should be noted that roots which grew in soil receiving only air also had areas of dead roots.

Then if there is some injury sustained by the roots receiving natural gas treatment does this injure the stem and leaves? Attempts have been made to answer this question. The total growth in length made from buds produced during the preceding season when the roots were gassed was greater than that made by the control trees (table I). The growth in diameter was also greater in the treated trees than in the controls (table II). Therefore, the injury to the roots has not decreased the growth of the tops.

One thing that was uppermost in mind, as some injury to the roots was observed, was what effect would this have on the absorption from a dry soil, as that which trees naturally encounter late in the summer. During the season of 1947 and 1948 the soils were permitted to dry and observations were made on the leaf color and leaf retention by the different trees. Again there was no indication that the experimental trees were unable to absorb all the water necessary to retain their leaves.

Summary

One might then summarize the information gained from the experiments by pointing out:

1. that concentrations of natural illuminating gas in air of from 2.5 to 4.0% cause some injury to roots as well as a decreased development of roots of the American elm;

2. but that this injury to the roots does not seem to influence the growth of the stem.

DEPARTMENT OF BOTANY
UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

LITERATURE CITED

1. GUSTAFSON, F. G. Is natural gas injurious to flowering plants? Plant Physiol. **19**: 551–558. 1944.
2. KRAMER, P. J. The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. Amer. Jour. Bot. **20**: 481–492. 1933.

UNIVERSITY OF MICHIGAN LIBRARIES

THE EFFECT OF IRRIGATION, NITROGEN LEVEL AND SEASON ON THE COMPOSITION OF CABBAGE

BYRON E. JANES[1]

Received February 13, 1950

Introduction

The use of irrigation in the production of vegetables in the eastern part of the United States has shown a very rapid increase in the past 4 or 5 years. Irrigation not only increases the total yield, but also in many cases, the quality of the crop produced is improved as well. Physiologists are interested in learning if such differences in quality are largely of a physical nature or if there are also some chemical differences between poor and good quality vegetables.

The author in a previous publication (**5**) presented data on the composition of beans grown with several rates of irrigation. The main difference was one of moisture content—beans growing without irrigation having a higher per cent. dry weight than those which were irrigated. On a dry weight basis, there were small differences in carbohydrate, organic nitrogen and potassium content. It is characteristic of fruits to be rather uniform in composition regardless of the conditions under which they are grown. It was felt that a crop which consists largely of leaf tissue would show more marked variations in composition associated with a difference in growth than do fruits. This study was made to determine the effect that (1) soil moisture (as influenced by irrigation), (2) nitrogen level and (3) season had on the composition of cabbage and also if there was any relation between quality and chemical nature of the crop. Quality is used here to mean appearance, succulence and similar factors which made for high consumer acceptance.

Materials and methods

Cultural practices

Cabbage of the variety Glory of Enkhuizen, was grown on the experimental plots of the Florida Agricultural Experiment Station at Gainesville, Florida, with four levels of water supply (three rates of irrigation and no irrigation), two levels of nitrogen fertilization and in two different seasons. The plantings were made in a Latin square, the main plot (irrigation) split two ways, first by time of planting and then by amount of nitrogen. The irrigation treatments were applied by overhead sprinklers which covered an area with a radius of 30 feet. The plots were 40 feet square. Cabbage plants were planted one foot apart in rows three feet apart. Each planting consisted of three rows with a guard row between. Three plantings were

[1] Formerly Associate Horticulturist at the Florida Agricultural Experiment Station.

made, (1) October 20, 1947, (2) December 3, 1947 and (3) February 6, 1948. The December 3 planting was severely damaged by frost and no analysis was made of this crop. A ton per acre of a commercial fertilizer supplying 4% N., 7% P_2O_5 and 5% K_2O was applied in bands at the time of planting. Each planting was divided into two 20-foot subplots one of which received additional nitrogen in the form of $NaNO_3$ as a side dressing.

The first planting of cabbage was started in the relatively warm weather of October and harvested in the cool weather of December and January. This resulted in the cabbage maturing over a long period of time. The third planting was made in the cool weather of February and harvested in warm weather of April, resulting in uniform maturity. The cabbage harvested in April made excellent growth and was by far the best cabbage from a commercial viewpoint.

The three irrigation treatments were frequent, medium, and occasional. The fourth treatment was no irrigation. The original plan was to determine the time of irrigation by measuring the amount of evaporation from an open pan—frequent irrigation, a half inch of water applied after one quarter inch of evaporation, medium irrigation, a half inch of water applied after each half inch of evaporation and occasional irrigation, half an inch of water applied after one inch of evaporation. A rainfall of half an inch or more was considered as one irrigation and the amount of evaporation to determine next irrigation was measured from the end of the rain. This procedure for determining the time to apply irrigation was very satisfactory in the cool weather of the winter months when evaporation was rather slow; however, in the spring when the rate of evaporation increased, these intervals between irrigation became too short. The following schedule for irrigating the several plots was adopted.

A half inch of water was applied to the frequent plots every second day, to the medium plots every fourth day and to the occasional plots every eighth day. A half inch or more of rain was considered as one irrigation and the time of next application dated from the day of rain. Table I gives the rainfall and amount of irrigation applied.

The amount of soil moisture was determined on three different dates (table II) by the method of STOUT and HOLBEN (9). The amount of nitrate nitrogen in the soil was determined before and after applying one of the side dressings of nitrogen (table III) (soil nitrogen analyses were made by G. M. Volk, Soils Department, Florida Agricultural Experiment Station, Gainesville, Florida).

ANALYTICAL METHODS

The cabbage was harvested when the heads were firm. A record was kept of the total number and weight produced on each plot. A sample consisting of ten heads from each plot was taken to the laboratory and subsamples obtained for the several analytical procedures. The cabbage heads

TABLE I

TOTAL AMOUNT OF WATER, RAINFALL PLUS IRRIGATION, FOR THE VARIOUS IRRIGATION TREATMENTS OF CABBAGE (IN INCHES)

	RAINFALL NO IRRIGATION	OCCASIONAL IRRIGATION AND RAINFALL	MEDIUM IRRIGATION AND RAINFALL	FREQUENT IRRIGATION AND RAINFALL
First planting October 20—January 10	7.59	8.09	9.09	15.09
Third planting February 9–April 29	13.00	14.50	18.00	23.00

were washed in tap water, the excess water removed by shaking and then wiping with cheesecloth. A quarter of each head was chopped into pieces one-fourth to one-half inch square, thoroughly mixed and samples for the various analyses to be made taken from the composited material. Dry weight was determined by drying two 100 gram samples in forced draft at 70° C. Separate 25 gram samples were used for ascorbic acid and carbohydrate determinations. Three hours or less elapsed from the time the cabbage was harvested until the sampling of the fresh material was completed. Ascorbic acid was determined by a modification of the Morell method (**6**). The sample for carbohydrate analysis was preserved by heating in boiling 80% alcohol for 45 minutes. The preserved material was ground to a very fine suspension in a Waring Blendor and made to definite volume with 80% ethyl alcohol. An aliquot of the suspension was centrifuged and the clear liquid used for determination of reducing sugars. The

TABLE II

SOIL MOISTURE PERCENTAGE OF THE INDIVIDUAL PLOTS ON THREE DIFFERENT DATES

PLOT NO.	IRRIGATION TREATMENT	10-24-47	12-2-47	4-28-48
1A		11.4	11.0	10.4
3B		10.9	7.9	8.8
2C	Frequent	11.7	8.4	8.4
4D		10.3	7.1	8.8
4A		.....	6.8	6.8
2B		.....	6.6	8.0
3C	Medium	.....	5.4	7.2
1D		.....	5.7	7.2
3A		.....	5.9	3.8
4B		.....	6.1	3.8
1C	Occasional	.....	5.8	4.0
2D		.....	4.4	4.2
2A		.....	7.3	3.6
1B		.....	6.2	5.2
4C	None	.....	5.2	3.8
3D		.....	5.2	3.0

residue was hydrolyzed with 10% HCl at 15 pounds pressure for one hour and the reducing power of the neutralized solution determined. The dry weight of the acid insoluble residue was determined. The reducing power of the various solutions was determined by the Shafer-Somogyi method as modified by HEINZE and MURNEEK (4). The material dried for moisture determination was ground to pass a 40-mesh screen and used for nitrogen and mineral analysis. The micro-Kjeldahl method was used for organic nitrogen. A sample of the dried material was ashed at 450° C in a stream

TABLE III

THE EFFECT OF LOCATION IN THE FIELD, SIDE DRESSING WITH $NaNO_3$ AND IRRIGATION ON THE AMOUNT OF NITRATE IN THE SOIL. SAMPLES TAKEN FROM OCTOBER PLANTING

PLOT NO.	IRRIGATION TREATMENT	P.P.M. OF NITRATE ON 12-19-47		P.P.M. OF NITRATE ON 12-22-47
		SIDE DRESSING	NOT SIDE DRESSED	AFTER SECOND SIDE DRESSING AND FREQUENT IRRIGATION*
1A	Frequent	1.0	1.0	46.0
3B		1.3	1.2	26.0
2C		1.0	1.0	13.0
4D		1.0	1.0	46.0
Average	Frequent	1.0	1.0	32.8
4A	Medium	1.0	1.0	82.8
2B		.8	1.2	105.0
3C		1.0	1.0	57.5
1D		1.0	1.0	33.1
Average	Medium	1.0	1.0	69.6
3A	Occasional	8.3	1.0	—
4B		6.0	2.0	—
1C		2.3	1.0	—
2D		1.0	1.0	—
Average	Occasional	4.4	1.2	—
2A	None	8.8	1.0	—
1B		2.5	1.3	—
4C		1.0	1.0	—
3D		1.0	1.0	—
Average	None	3.3	1.0	—

* The second side dressing was applied to all plots and water applied only to the frequent plots on 12-20-47. A half inch of rain fell on 12-21-47.

of oxygen, the ash dissolved in 10% HCl and the amount of calcium, magnesium, potassium, sodium, sulphate, and phosphate in the solution determined.

Calcium was precipitated as the oxalate and titrated with standardized $KMnO_4$ solution. Magnesium was precipitated as magnesium ammonium phosphate and titrated with standard acid. Potassium was determined by precipitating as cobaltinitrite and then titrating with ceric sulphate as described by BROWN (2). Sodium was precipitated by the method of BARBER and KOLTHOFF (1) using uranyl-zinc acetate. Phosphate was determined

colorimetrically by the molybdate blue method of TRUOG and MEYER (**10**). Sulphate was determined by the barium chromate method of FOSTER (**3**).

Separate samples of the dried material were used for iron and manganese determination. Iron was determined by ortho-phenanthroline method of Sawell and Cunningham as modified by SHEETS and WARD (**7**). Manganese was determined according to procedure described by SOMERS and SHIVE (**8**). The amount of iron contamination arising from grinding in the Wiley mill was found to be negligible when proper methods were used in cleaning the mill before use.

Results

The rainfall (table I) during the growth of the first planting was less than that during the growth of the third planting. However, due to the fact that it was cool and the rainfall was evenly spaced during much of the first period little additional irrigation was required. Most of the rain for the third planting came early in the season so that during the time of rapid growth there was an extended dry period. The average weight of the heads of cabbage (tables IV and V) reflect the variation in amount of moisture available for growth. The cabbage harvested in January showed no response to additional water. However, despite the much higher total rainfall during the growth of the third crop there was a marked response to irrigation.

The data of table II and III are included to show some of the variation in soil moisture and nitrate level in various parts of the field. The soil ranged in texture from quite sandy (tiers C and D) to soil with some clay (tiers A and B). There was a uniform irrigation treatment given just prior to samples taken October 24, 1947 and these figures represent the variation in the moisture-holding capacity of the soil. The samples taken on December 2, 1947 and April 28, 1948 were secured just prior to applying water to all three treatments. The December 2, 1947 samples were taken during one of the short dry periods in the growth of the first crop. The evaporation rates were low and there were only small differences in soil moisture due to the differential irrigation treatments. There was as much difference due to location in the field as to amount of irrigation applied. The April 28, 1948 samples were taken after a considerable period of dry weather during which the evaporation rate was high and the plants were quite large. These values represent the maximum range in soil moisture which were obtained during the growth of the crop.

The nitrate was quite easily leached from the soil especially on the more sandy areas. The data of table III show that the nitrate was leached most rapidly by the frequent irrigation and that side dressing with $NaNO_3$ did help to maintain the nitrate level in the soil.

TABLE IV

THE EFFECT OF IRRIGATION ON THE COMPOSITION OF CABBAGE HARVESTED IN JANUARY

CONSTITUENT	FREQUENT IRRIGATION	MEDIUM IRRIGATION	OCCASIONAL IRRIGATION	NO IRRIGATION	DIFFERENCE NECESSARY FOR SIGNIFICANCE AT ODDS OF	
					19 to 1	99 to 1
	Fresh weight					
Weight per head (lbs.)	1.4	1.7	1.5	1.5	N.S.*	N.S.*
Dry weight (per cent.)	8.3	8.1	8.2	8.0	.29	.45
Ascorbic acid (mgs. per 100 gms.)	52.8	50.4	50.1	52.9	N.S.	N.S.
Reducing sugars (per cent.)	3.4	3.4	3.5	3.5	N.S.	N.S.
Acid hydrolyzable carbohydrates (per cent.)	.74	.71	.77	.75	N.S.	N.S.
Acid insoluble residue (per cent.)	.66	.69	.71	.69	N.S.	N.S.
Ash (per cent.)	.69	.66	.70	.67	N.S.	N.S.
Calcium (per cent.)	.084	.077	.085	.090	.012	.018
Magnesium (per cent.)	.014	.014	.014	.014	N.S.	N.S.
Potassium (per cent.)	.26	.25	.26	.25	N.S.	N.S.
Sodium (per cent.)	.014	.016	.020	.017	N.S.	N.S.
Phosphate (per cent.)	.100	.093	.106	.090	.010	.016
Nitrogen (per cent.)	.204	.192	.206	.221	.024	.037
Sulphate (per cent.)	.095	.089	.093	.089	N.S.	N.S.
Iron (mgs. per 100 gms.)	.29	.24	.29	.27	N.S.	N.S.
Manganese (mgs. per 100 gms.)	.14	.19	.20	.20	N.S.	N.S.
	Dry weight					
Ascorbic acid (mgs. per 100 gms.)	641	626	613	662	N.S.	N.S.
Reducing sugars (per cent.)	40.6	42.0	42.4	43.2	N.S.	N.S.
Acid hydrolyzable carbohydrates (per cent.)	9.5	8.8	9.4	9.4	N.S.	N.S.
Acid insoluble residue (per cent.)	8.0	8.5	8.7	8.6	N.S.	NS.
Ash (per cent.)	8.3	8.1	8.6	8.4	N.S.	N.S.
Calcium (per cent.)	1.03	.97	1.04	1.13	N.S.	N.S.
Magnesium (per cent.)	.16	.17	.18	.18	N.S.	N.S.
Potassium (per cent.)	3.16	3.06	3.15	3.16	N.S.	N.S.
Sodium (per cent.)	.17	.21	.25	.21	.07	.11
Phosphate (per cent.)	1.16	1.16	1.29	1.13	.12	.18
Nitrogen (per cent.)	2.47	2.39	2.51	2.77	.31	.46
Sulphate (per cent.)	1.15	1.10	1.14	1.11	N.S.	NS.
Iron (mgs. per 100 gms.)	3.44	2.98	3.50	3.40	N.S.	N.S.
Manganese (mgs. per 100 gms.)	1.69	2.34	2.44	2.47	.65	.98

* N.S. indicates no significance.

IRRIGATION

There was no differential response in the growth of the plants harvested in January and as would be expected, there was no major difference in the composition of the cabbage (table IV) either on a fresh weight or dry weight basis.

Because of the dry weather during the period of rapid growth of the cabbage harvested in April, the differential irrigation treatments resulted in large differences in growth. The cabbage receiving no additional water averaged 1.5 pounds per head while those receiving frequent irrigation averaged 2.7 pounds per head. This marked difference in growth was reflected in the composition. On a fresh weight basis, all the constituents

TABLE V

THE EFFECT OF IRRIGATION ON THE COMPOSITION OF CABBAGE HARVESTED IN APRIL

CONSTITUENT	FREQUENT IRRIGATION	MEDIUM IRRIGATION	OCCASIONAL IRRIGATION	NO IRRIGATION	DIFFERENCE NECESSARY FOR SIGNIFICANCE AT ODDS OF	
					19 to 1	99 to 1
	Fresh weight					
Weight per head (lbs.)	2.7	2.5	1.8	1.5	.29	.45
Dry weight (per cent.)	6.8	7.2	8.1	8.5	.37	.56
Ascorbic acid (mgs. per 100 gms.)	40.9	44.6	53.7	55.5	2.71	4.11
Reducing sugars (per cent.)	2.9	3.0	3.1	2.9	.17	.26
Acid hydrolyzable carbohydrates (per cent.)	.58	.67	.83	.84	.13	.20
Acid insoluble residue (per cent.)	.63	.65	.73	.78	.12	.18
Ash (per cent.)	.56	.57	.66	.71	.039	.059
Calcium (per cent.)	.075	.072	.083	.098	.0095	.0143
Magnesium (per cent.)	.0083	.0091	.010	.012	.0021	.0032
Potassium (per cent.)	.20	.19	.23	.25	.0212	.0321
Sodium (per cent.)	.023	.030	.025	.026	.0086	.0131
Phosphate (per cent.)	.085	.087	.106	.099	.017	.026
Nitrogen (per cent.)	.14	.16	.21	.24	.027	.041
Sulphate (per cent.)	.09	.11	.11	.12	.027	.041
Iron (mgs. per 100 gms.)	.24	.27	.30	.38	.082	.124
Manganese (mgs. per 100 gms.)	.12	.14	.17	.17	.027	.041
	Dry weight					
Ascorbic acid (mgs. per 100 gms.)	603	620	669	660	11.9	18.1
Reducing sugars (per cent.)	42.4	41.8	38.8	34.3	4.04	6.11
Acid hydrolyzable carbohydrates (per cent.)	8.6	9.4	10.3	10.0	1.33	2.03
Acid insoluble residue (per cent.)	9.4	9.0	9.1	9.3	N.S.	N.S.*
Ash (per cent.)	8.3	8.0	8.2	8.5	N.S.	N.S.
Calcium (per cent.)	1.10	.99	1.04	1.17	N.S.	N.S.
Magnesium (per cent.)	.12	.13	.13	.15	.02	.04
Potassium (per cent.)	2.90	2.62	2.82	3.02	.20	.31
Sodium (per cent.)	.34	.41	.31	.31	.08	.12
Phosphate (per cent.)	1.25	1.21	1.31	1.18	N.S	N.S.
Nitrogen (per cent.)	2.10	2.30	2.40	2.90	.23	.35
Sulphate (per cent.)	1.33	1.27	1.36	1.43	.08	.13
Iron (mgs. per 100 gms.)	3.25	3.76	3.77	4.52	1.0	1.5
Manganese (mgs. per 100 gms.)	1.81	1.77	2.09	1.95	N.S.	N.S.

* N.S. indicates no significance.

measured except sodium were lowest in the cabbage grown with frequent irrigation and as the frequency of irrigation was reduced, the percentage of each constituent increased. There was no difference in the sodium content on a fresh weight basis associated with different irrigation treatments.

That this difference in composition is to a large extent one of hydration is shown by the fact that on a dry weight basis several of the differences disappear and most of them are less significant. It is interesting to note that on a dry weight basis, the reducing sugar decreased with a decrease in amount of water applied, but acid hydrolyzable carbohydrates increase. The increase in the acid hydrolyzable carbohydrate is much smaller than

the decrease in reducing sugars so that the net effect is one of reduction of carbohydrate as irrigation is reduced. The per cent. of nitrogen increased with a decrease in frequency of irrigation.

The mineral elements are not as consistent in their variation as the organic constituents. This is shown by the percentages of potassium, sodium, and sulphate in the cabbage grown with medium irrigation. Both potassium and sulphate are lower in the medium samples than in the others and sodium is higher. The percentage of magnesium and iron on a dry weight basis increased with decrease in irrigation. The differences in percentage of ash, calcium, phosphate and manganese are not significant.

Nitrogen level

Apparently the leaching of nitrate was greater during the growth of the first planting of cabbage than during the last. There was an increase in size of cabbage heads with increase in nitrogen in the cabbage harvested in January, but there was no difference in the cabbage harvested in April. However, it is interesting to note that there were more differences in composition in the April harvest than in the January harvest. In both harvests, the greatest difference was in the sodium content. The cabbage receiving the additional $NaNO_3$ had a much higher sodium content than that not side dressed. This was the only significant difference in the cabbage harvested in January. In the April harvest, an increase in amount of nitrogen added to the soil was associated with an increase in the amount of nitrogen and a decrease in the amount of carbohydrate in the plant. On a dry weight basis, additional side dressings of $NaNO_3$ increased the percentage of phosphate.

Season

There were a few small differences in composition between the January and April harvests. These differences were largely associated with the difference in response of the two crops to irrigation treatments. The composition of the non-irrigated cabbage harvested in April was very similar to the cabbage harvested in January. However, the samples taken from the frequent irrigation treatments in April had larger heads, less dry weight and in general a smaller percentage of most of the constituents than did the cabbage harvested in January.

Discussion

The primary purpose of this study was to obtain information on the difference in composition of irrigated and non-irrigated crops to help in the evaluation of irrigation in the production of vegetables. In addition, as was pointed out in the introductory statement, an attempt was also made to determine if there was any correlation between quality and chemical composition. Very little information on this latter point was obtained largely because there was little difference in what could be termed market quality

TABLE VI

EFFECT OF NITROGEN LEVEL ON THE COMPOSITION OF CABBAGE GROWN AT FOUR LEVELS OF SOIL MOISTURE—HARVESTED IN JANUARY

CONSTITUENT	FRESH WEIGHT				DRY WEIGHT			
	Two side-dressings of $NaNO_3$	No side-dressings	Difference necessary for significance at odds of		Two side-dressings of $NaNO_3$	No side-dressings	Difference necessary for significance at odds of	
			19 to 1	99 to 1			19 to 1	99 to 1
Weight per head (lbs.)	1.6	1.3	.21	.29				
Dry weight (per cent.)	7.9	8.3	.27	.37				
Ascorbic acid (mgs. per 100 gms.)	51.0	52.1		N.S.*	638	632		N.S.
Reducing sugars (per cent.)	3.4	3.4		N.S.	42.4	41.8		N.S.
Acid hydrolyzable carbohydrates (per cent.)	.77	.72		N.S.	9.2	9.4		N.S.
Acid insoluble residue (per cent.)	.67	.70		N.S.	8.4	8.4		N.S.
Nitrogen (per cent.)	.21	.20		N.S.	2.64	2.43		N.S.
Ash (per cent.)	.68	.68		N.S.	8.46	8.29		N.S.
Calcium (per cent.)	.082	.086		N.S.	1.04	1.05		N.S.
Magnesium (per cent.)	.014	.014		N.S.	.18	.17		N.S.
Potassium (per cent.)	.25	.26	.006	.008	3.12	3.15		N.S.
Sodium (per cent.)	.023	.011	.003	.005	.28	.14	.03	.04
Phosphate (per cent.)	.099	.100		N.S.	1.23	1.13	.09	.13
Sulphate (per cent.)	.089	.094	.004	.006	1.14	1.16		N.S.
Iron (mgs. per 100 gms.)	.27	.27		N.S.	3.35	3.31		N.S.
Manganese (mgs. per 100 gms.)	.18	.18		N.S.	2.25	2.22		N.S.

* N.S. indicates no significance.

TABLE VII

EFFECT OF NITROGEN LEVEL ON THE COMPOSITION OF CABBAGE GROWN AT FOUR LEVELS OF SOIL MOISTURE—HARVESTED IN APRIL

CONSTITUENT	FRESH WEIGHT				DRY WEIGHT			
	FOUR SIDE-DRESSINGS OF $NaNO_3$	NO SIDE-DRESSINGS	DIFFERENCE NECESSARY FOR SIGNIFICANCE AT ODDS OF		FOUR SIDE-DRESSINGS OF $NaNO_3$	NO SIDE-DRESSINGS	DIFFERENCE NECESSARY FOR SIGNIFICANCE AT ODDS OF	
			19 to 1	99 to 1			19 to 1	99 to 1
Weight per head (lbs.)	2.1	2.1		N.S.*				
Dry weight (per cent.)	7.5	7.7		N.S.				
Ascorbic acid (mgs. per 100 gms.)	49.0	48.3		N.S.	654	621	5.2	7.2
Reducing sugars (per cent.)	2.8	3.2	.17	.23	37.6	41.0	2.6	3.6
Acid hydrolyzable carbohydrates (per cent.)	.65	.81	.09	.13	8.7	10.4	1.1	1.5
Acid insoluble residue (per cent.)	.66	.73	.05	.08	8.9	9.5	.56	.77
Nitrogen (per cent.)	.20	.18	.011	.015	2.6	2.4	N.S.	
Ash (per cent.)	.63	.62		N.S.	8.44	8.05	.28	.38
Calcium (per cent.)	.083	.080		N.S.	1.11	1.04	N.S.	
Magnesium (per cent.)	.010	.009		N.S.	.13	.13	N.S.	
Potassium (per cent.)	.21	.22		N.S.	2.85	2.84	N.S.	
Sodium (per cent.)	.031	.019	.0021	.0029	.43	.25	.03	.04
Phosphate (per cent.)	.097	.091		N.S.	1.30	1.18	.064	.088
Sulphate (per cent.)	.11	.10		N.S.	1.35	1.35	N.S.	
Iron (mgs. per 100 gms.)	.30	.30		N.S.	3.98	3.81	N.S.	
Manganese (mgs. per 100 gms.)	.15	.15		N.S.	1.95	1.93	N.S.	

* N.S. indicates no significance.

even though there was considerable difference in growth. If appearance and succulence are used as a basis for quality, the cabbage harvested from the frequent irrigation plots in April had the highest quality. The greatest difference between the best quality and the poorer cabbage was in moisture content. There were other differences but most of them were so small that it would require much more data before any definite correlation could be made. This information would indicate that perhaps the physical characteristics of the plant are more important than composition in determining quality. In this connection it might be of value to make such measurements as the size of cells and the relationship of one tissue to another.

Summary

Cabbage was grown with four levels of water supply—frequent, occasional, medium irrigation and without supplemental water—two levels of nitrogen fertilization and in two different seasons. Samples of cabbage were analyzed for dry weight, ascorbic acid, reducing sugars, acid hydrolyzable carbohydrates, acid insoluble residue, ash, calcium, magnesium, potassium, sodium, phosphate, nitrogen, sulphate, iron, and manganese.

There was no difference in growth or composition of cabbage harvested from the various irrigation treatments in January.

Because of an extended period of dry weather during the growth of the cabbage harvested in April there was a marked response in growth of the crop on different plots. Associated with this difference in growth were the following differences in composition. On a fresh weight basis, all constituents estimated except sodium were highest in the non-irrigated cabbage and lowest in the frequently irrigated cabbage. On a dry weight basis the reducing sugars decreased and acid hydrolyzable carbohydrates and nitrogen increased with a decrease in the amount of water added. On a dry weight basis sulphate and potassium are lower and sodium is higher in the medium samples. The percentage of iron and manganese increase with a decrease in irrigation.

The main effect of side dressing with $NaNO_3$ was to increase the sodium content.

The only difference between seasons was associated with difference in response to irrigation treatments.

UNIVERSITY OF CONNECTICUT
STORRS, CONNECTICUT

LITERATURE CITED

1. BARBER, H. H. and KOLTHOFF, I. M. A specific reagent for the rapid gravimetric determination of sodium. Jour. Amer. Chem. Soc. **50**: 1625–1631. 1928.
2. BROWN, D. S., ROBINSON, R. R. and BROWNING, G. M. Determination of small amounts of potassium. Ind. and Eng. Chem. Anal. Ed. **10**: 652–654. 1938.

3. Foster, M. D. Volumetric determination of sulfate by barium chromate method. Ind. and Eng. Chem. Anal. Ed. **8**: 195. 1936.
4. Heinze, P. H. and Murneek, A. E. Comparative accuracy and efficiency in determination of carbohydrates in plant material. Research Bul. 314. Missouri Agr. Exp. Sta. 1940.
5. Janes, B. E. The effect of varying amounts of irrigation on the composition of two varieties of snap beans. Proc. Amer. Soc. Hort. Sci. **51**: 457–462. 1948.
6. Morell, S. A. Rapid photometric determination of ascorbic acid in plant materials. Ind. and Eng. Chem. Anal. Ed. **13**: 793–794. 1941.
7. Sheets, O. A. and Ward, M. V. Studies in nutritional anemia. Mississippi Agr. Exp. Sta. Tech. Bul. 26. 1940.
8. Somers, I. I. and Shive, J. W. The iron and manganese relation in plant metabolism. Plant Physiol. **17**: 582–602. 1942.
9. Stout, G. J. and Holben, F. J. Simple method for rapid drying of soil moisture. Proc. Amer. Soc. Hort. Sci. **47**: 238. 1946.
10. Truog, E. and Meyer, A. A. Improvements in the Deniges colorimetric method for phosphorous and arsenic. Ind. and Eng. Chem. Anal. Ed. **1**: 136–139. 1929.

THE EFFECTS OF FLOODING ON THE TRANSPIRATION AND SURVIVAL OF SOME SOUTHEASTERN FOREST TREE SPECIES

JOHNSON PARKER

Received July 25, 1949

Field observations indicate that the distribution of certain forest tree species is modified by periodic flooding of the soil. There appear to be distinct differences between species with respect to the amount of flooding which they will tolerate. Overcup oak, for example, often occurs on areas which are flooded for considerable periods of time during the year, but northern red oak usually occurs only on the better drained sites. Cypress commonly grows on flooded soil, but loblolly pine seldom occurs in such wet locations. Presumably the roots of some species are more susceptible to injury from the lack of oxygen and excess of carbon dioxide found in flooded soils, and injury to the roots usually results in decreased absorption of water and minerals and in damage to the shoots. Since very few measurements of the effect of flooding the soil on forest trees have been made, a study was planned on potted seedlings.

It was assumed that injury to the roots by flooding would be indicated by a decrease in transpiration resulting from decreased water absorption. Measurement of transpiration should therefore indicate the extent of injury to the roots caused by flooding.

The species used and their origin were as follows: Loblolly pine, *Pinus taeda,* L., North Carolina. Red cedar, *Juniperus virginiana,* L., North Carolina. Cypress, *Taxodium distichum,* (L.) Richard, South Carolina. White oak, *Quercus alba,* L., North Carolina. Swamp chestnut oak, *Quercus prinus,* L., Tennessee. Red oak, *Q. borealis,* var. maxima (Marsh.) Ashe, North Carolina. Overcup oak, *Q. lyrata,* Walt., Tennessee. Dogwood,*Cornus florida,* L., North Carolina.

All of these seedlings except cypress had been grown out-of-doors in clay pots for two years and were about 16 inches tall. The cypress was brought from the Coastal Plain about four months before the experiments were started and kept out-of-doors in clay pots until used.

Methods

The soil masses containing the root systems of the experimental plants were transferred from clay pots in which they had been growing to metal cans painted with asphaltum on the inside and white enamel on the outside. Tops of all cans were covered with two layers of oilcloth which had been cut so that the cloth might be placed around the stems of the plants. Seedlings of each species were tested in groups of six, three individuals

being flooded and three kept in soil at approximately field capacity as controls. An exception to this procedure was the last experiment in which only one species was used as a control. Previous work indicated that the principal difference in daily transpiration rate between different species of forest tree seedlings kept in soil at field capacity is largely accounted for by differences in the root and leaf surface (PARKER, 6). It thus seemed sufficient in this experiment to use only one species as a control.

After a preliminary test period during which transpiration rates were measured every 24 hours, the soil of three plants of each species was saturated with tap water so that the water level stood about two inches above the soil surface. About every three days flooded containers were opened at the tops and water added to maintain the levels. Control containers were watered approximately every two days to maintain the soil at field capacity. After one month of flooding containers were drained by allowing them to lie on their sides with covers removed for a period of 12 hours. Transpiration rates were measured by successive weighings of containers at

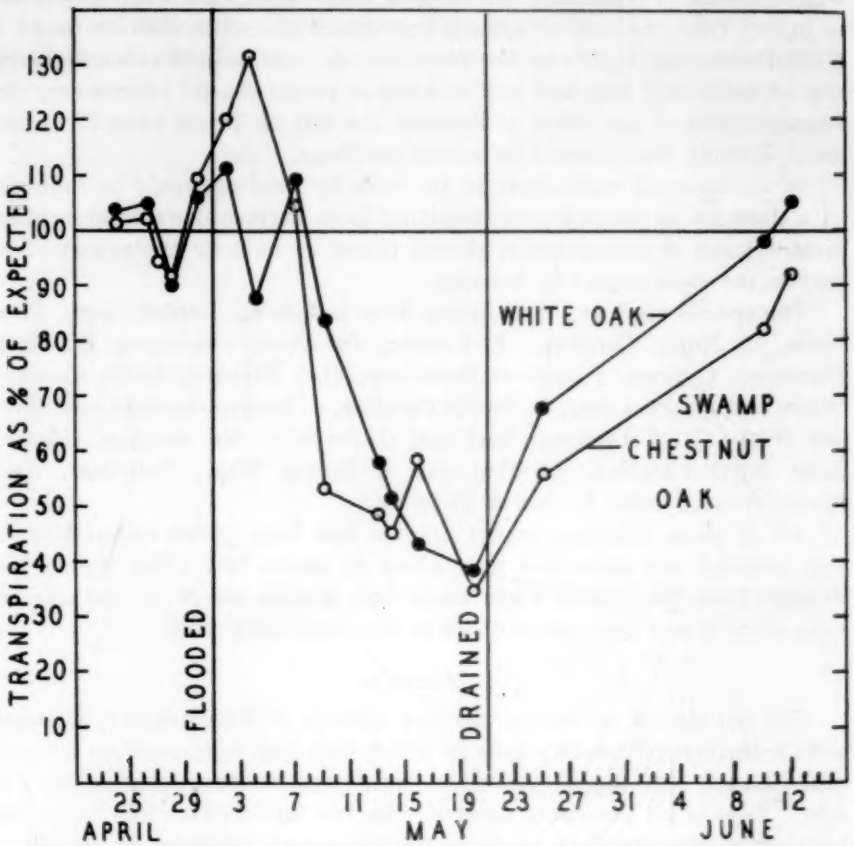

FIG. 1. Transpiration of white oak and swamp chestnut oak seedlings in flooded soil plotted as percentages of the expected rates. Spring 1948.

24-hour intervals. All plants were kept in the greenhouse and were distributed at random on a shelf under unshaded glass.

Transpiration rates are expressed as a percentage of the expected rate somewhat according to the method of GRADMANN (3). The behavior of the treated and control plants is compared before flooding and a factor derived which may be applied to the control rates at any time during the experiment to find the "expected" rate of the treated plants. The expected rate is calculated, therefore, on the basis of the pre-flooding behavior and on the continued behavior of the control plants throughout the experiment. Values plotted are the average daily values of the flooded plants divided by the average expected rates for each day, times 100.

Results

The effects on transpiration of flooding seedlings of white oak and swamp chestnut oak in the spring of 1948 are shown in figure 1. Flooding resulted in a decline in transpiration which was essentially the same for both species. Recovery was also similar for both species. There was, therefore, no evidence that swamp chestnut oak tolerates flooding in closed containers better than does white oak.

Results of flooding eastern red cedar, red oak, and dogwood in the

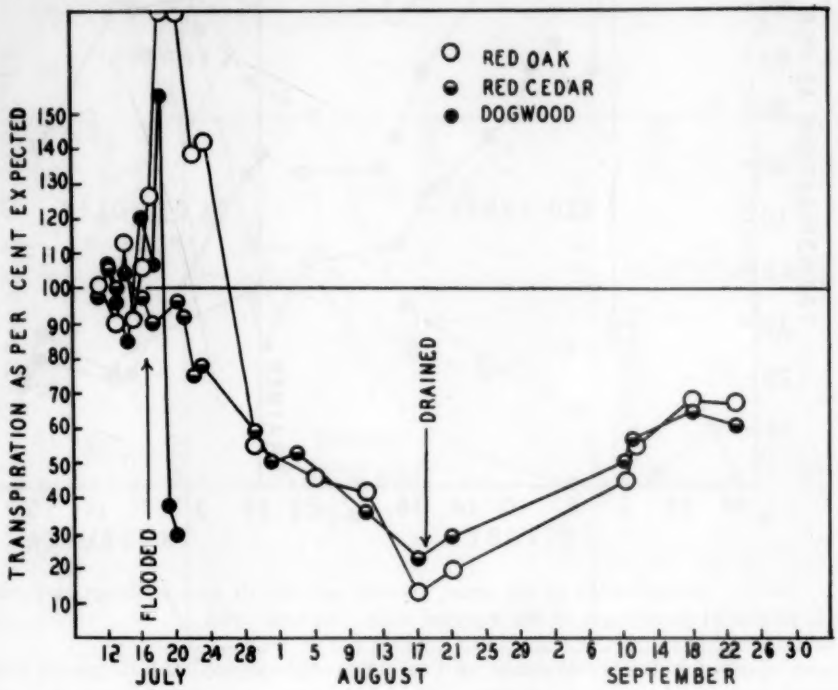

FIG. 2. Transpiration of red oak, red cedar, and dogwood seedlings in flooded soil plotted as percentages of the expected rates. Summer 1948.

summer of 1948 are shown graphically in figure 2. There was little or no difference between red cedar and red oak in their reaction to flooding. Both species declined in transpiration at a rate similar to that of white oak and swamp chestnut oak shown in figure 1. The leaves of dogwood, how-

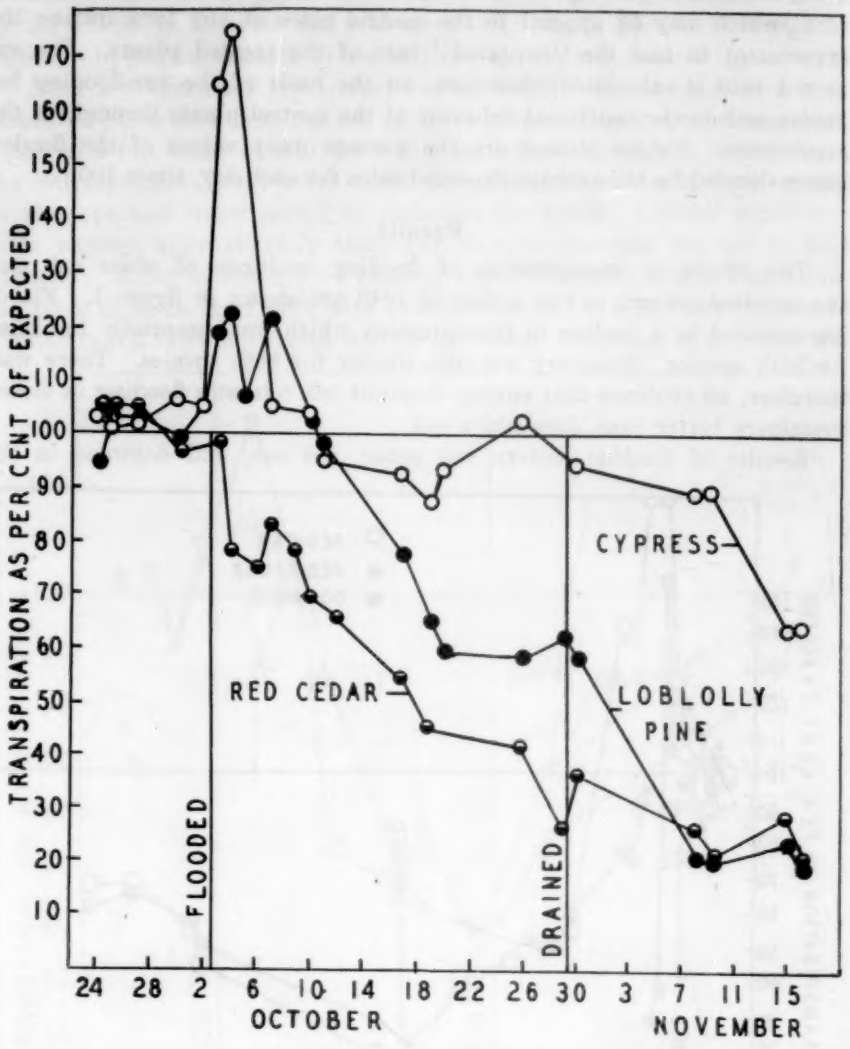

FIG. 3. Transpiration of red cedar, cypress, and loblolly pine seedlings in flooded soil plotted as percentages of the expected rates. Autumn 1948.

ever, showed a discoloration after two days of flooding. At the same time transpiration decreased rapidly. Leaves became a mottled reddish color on the third day after flooding, then curled and dropped off. A single bud on one dogwood opened after flooding and its leaves remained healthy

in appearance for about three weeks, but died shortly afterwards. Another set of three dogwoods was flooded in the summer of 1949, but all leaves did not die for almost three weeks after flooding. Weather was about the same in both experiments. One month after drainage oaks and cedars recovered to about 60% of the expected rate. It is probable that two months after drainage enough new roots would have developed to bring the absorption rate back to the expected level as indicated by the transpiration rate.

The effect of flooding loblolly pine, red cedar, and cypress in the autumn of 1948 is shown graphically in figure 3. Red cedar suffered most rapidly from flooding and loblolly pine suffered only slowly at first. Cypress continued at about the expected normal rate after a temporary high rate in the first two days of flooding. After drainage, transpiration of loblolly pine fell to the same level as that of red cedar. The cause of this is not clear. The decline of the transpiration of cypress was believed to be associated with the exposure of roots to air that had grown into the water layer above

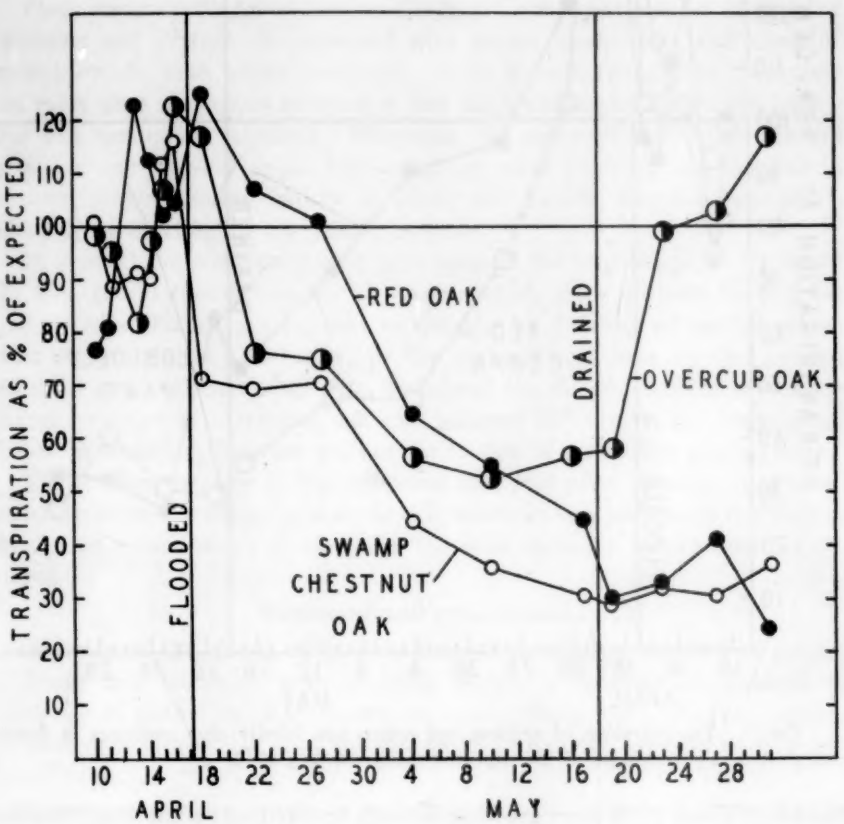

FIG. 4. Transpiration of red oak, swamp chestnut oak, and overcup oak seedlings in flooded soil plotted as percentages of the expected rates. Spring 1949.

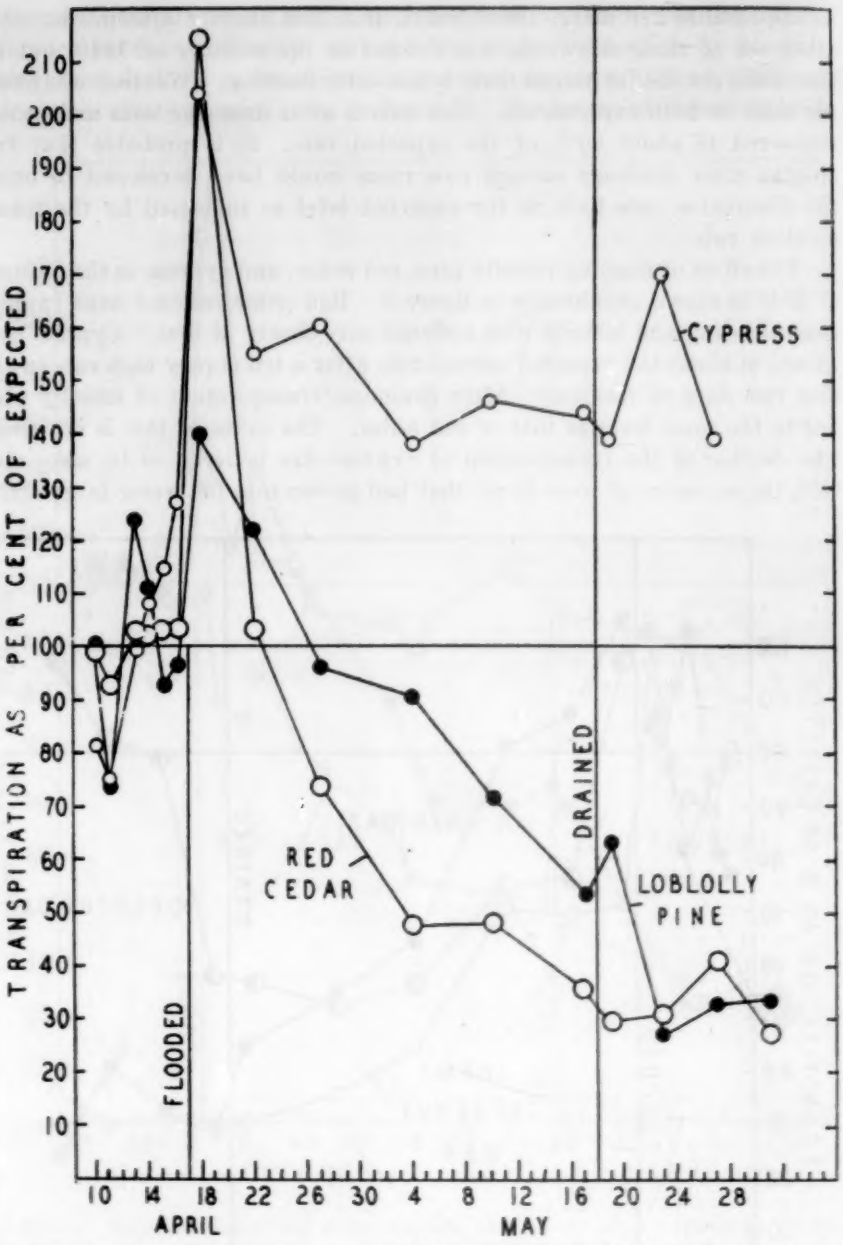

FIG. 5. Transpiration of cypress, red cedar, and loblolly pine seedlings in flooded soil plotted as percentages of the expected rates. Spring 1949.

the soil. These roots were growing directly upward into what was presumed to be the better aerated water at the surface.

An attempt was made to duplicate the results of the previous experi-

ments in one further experiment. This was carried out in the spring of 1949 using red cedar, loblolly pine, cypress, red oak, swamp chestnut oak, and overcup oak. Results are shown graphically in figures 4 and 5. Loblolly pine and red cedar behaved as they had in the previous experiment. The increase in transpiration rate of cypress before flooding may be related to rapid root growth after being transferred from the clay pots to the cans containing a small additional amount of new soil. At least, there was no definite decline as was observed with the other species.

All oaks behaved somewhat similarly but about four days before the end of the flooding period overcup oak seedlings began to produce a second crop of leaves, the first having appeared earlier that spring before the experiment was started. By the end of the experiment the second crop of leaves was almost fully developed, and undoubtedly this accounts in part for the increase in transpiration during the period just before and after the time of drainage.

Discussion

These results with forest tree seedlings are somewhat similar to those of CHILDERS and WHITE (**2**) obtained with young apple trees and those of LOUSTALOT (**5**) with pecan seedlings. These writers found that transpiration rates were sometimes reduced in two days, although a somewhat longer time was commonly required. HEINICKE (**4**) observed that when the soil in which one-year-old apple tree seedlings were growing was flooded in summer, leaves showed injury in about one month, but no damage resulted from flooding in the winter months.

In general the results reported here support the conclusions of CAUGHEY (**1**) who found that a number of species which grow on soils flooded for long periods of time are injured as quickly by flooding as species which grow on drier sites. However, in the experiments done by the present writer, a much greater ability to withstand the flooded condition was exhibited by cypress. Overcup oak also behaved differently, but its original decline of transpiration rate was similar to that of most other species tested.

The sudden increase in transpiration rate just after flooding in a number of experiments suggests that the soil moisture was somewhat limiting to absorption even though it was near the field capacity before flooding occurred.

Summary and conclusions

A study of the effects of flooding the soil in which a number of species of forest tree seedlings were growing showed a considerable amount of similarity in reduction of transpiration rate. There were, however, certain differences exhibited between species which ordinarily grow in widely different sites.

Cypress seedlings showed an outstandingly high level of transpiration rate after flooding, although there was evidence that the roots did not grow as well in the more poorly aerated levels of the container as in the better

aerated levels. Overcup oak showed a decline similar to the other oaks in the first few days of flooding, but unlike the other oaks, produced a second crop of leaves.

Red cedar, red oak, loblolly pine, white oak, and swamp chestnut oak all showed a similar response to the flooding treatment. Leaves of dogwood seedlings died shortly after flooding in one experiment but when the experiment was repeated the leaves died only after three weeks of flooding.

The writer is indebted to Professor Paul J. Kramer for the techniques used and advice given in the course of the work.

DEPARTMENT OF BOTANY
DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA

LITERATURE CITED

1. CAUGHEY, MARY G. A physiological and ecological study of certain pocosin plants. Plant Physiol. **20**: 671–689. 1945.
2. CHILDERS, N. F. and WHITE, D. G. Influence of submergence of the roots on transpiration, apparent photosynthesis, and respiration of young apple trees. Plant Physiol. **17**: 603–618. 1942.
3. GRADMANN, HANS. Untersuchungen über die Abhängigkeit der Transspiration und des Welkens von den Wasserverhältnissen des Bodens. Jahrb. für wiss. Bot. **76**: 558–663. 1932.
4. HEINICKE, A. J. Effect of submerging roots of apple trees. Amer. Soc. Hort. Sci. Proc. **29**: 205–207. 1932.
5. LOUSTALOT, A. J. Influence of soil moisture conditions on apparent photosynthesis and transpiration of pecan leaves. Jour. Agr. Res. **71**: 519–532. 1945.
6. PARKER, JOHNSON. Effects of variations in the root-leaf ratio on transpiration rate. Plant Physiol. **24**: 739–743. 1949.

SOME OBSERVATIONS ON NITRITE FORMATION AND THE ABSORPTION OF NITROGEN BY CITRUS[1]

EZRA H. BITCOVER AND IRVIN W. WANDER

(WITH TWO FIGURES)

Received January 12, 1950

The culture of citrus in Florida is frequently associated with sandy soils which allow rapid leaching and lead to nitrogen deficiency symptoms in the trees. The application of commercial forms of nitrogen to such soils leads to the question of the efficiency with which citrus trees utilize this nitrogen. The present article deals with certain observations made on the absorption of nitrate, ammonium, and urea nitrogen by year-old budded citrus trees.

Methods

Twelve trees of Valencia orange budded on rough lemon root stock were set in coarse ($\frac{1}{4}$ to $\frac{1}{8}$ inch) silica gravel. The containers were concrete conduit tile measuring one foot inside diameter and five feet tall, and were supported by concrete blocks to a height one foot above the ground. The lower end of each container was plugged with concrete at an angle, with an orifice at the lower edge to allow for complete drainage. The interior of each container was painted with an inert emulsifiable asphalt compound. In front of each container was a 5-gallon glass carboy for the appropriate nutrient solution, which was delivered to the container through the orifice by compressed air and returned to the carboy by gravity (**6**).

The methods employed to analyze the amounts of the different forms of nitrogen were all colorimetric, using a Fisher Electrophotometer, and employing standard colorimetric procedures slightly adapted to the requirements of the solutions themselves. Urea nitrogen was determined by means of Xanthydrol reagent (**4, 5**). Ammonium nitrogen was determined with Nessler's reagent (**5, 9**). Nitrite nitrogen was determined with dimethylaniline reagent (**12**). Nitrate nitrogen was determined by means of brucine reagent (**5, 8, 9, 11**).

Results

The trees, after being severely pruned of roots and shoots, were set in place May 7, 1948. Nutrient solutions were supplied starting May 10. The first leafy sprouts appeared after one week. The nutrient solutions were renewed at varying periods, depending on the amounts of the original forms of nitrogen changing to other forms of nitrogen, or the amounts

[1] This work was carried out at the University of Florida Citrus Experiment Station, Lake Alfred, Florida. Approved for publication by Director Mowry, Florida Agricultural Experiment Stations.

absorbed by the trees. The compositions of the nutrient solutions are in table I (**10**).

After three weeks it was noticed that one of the urea-supplied trees was wilting. During the succeeding several days the degree of wilting increased until after a week the tree showed signs of being severely wilted. Inspection showed that the tree was not wilting because of improper moisture and nutrient supply about its root system. It was decided that some

TABLE I

COMPOSITION OF THE NUTRIENT SOLUTIONS*

	MOLARITY	GMS./LITER	ML. OF 0.5 M 18 LITERS
Nitrate Solution			
KH_2PO_4	.001	0.136	36
$Ca(NO_3)_2$	.00225	.369	81
KNO_3	.0021	.212	75.6
$Mg(NO_3)_2$	.0043	.639	155
$MgSO_4$	.0014	.169	50.5
Ammonium Solution			
KH_2PO_4	.0031	.421	111.5
$MgSO_4$	.0057	.686	205
$CaSO_4$	.00225	.306	4050 (0.01 M.)
$(NH_4)_2SO_4$	.0076	.101	274
Urea Solution			
Urea	.0076	.456	274
KH_2PO_4	.0031	.421	111.5
$MgSO_4$	.0057	.686	205
$CaSO_4$	.00225	.306	4050 (0.01 M.)
⅓ Ammonium, ⅓ Nitrate, ⅓ Urea Solution			
$(NH_4)_2SO_4$	.0025	.034	91
Urea	.0025	.152	91
$Ca(NO_3)_2$	.00225	.369	81
KNO_3	.00057	.058	20.5
KH_2PO_4	.0025	.340	90
$MgSO_4$	.0057	.686	205

* Stock solutions of each of the salts were provided in 0.5 M. concentration and the culture solutions were made up in 18 liter lots with tap water. Boron and manganese salts, sufficient to provide 1 p.p.m. of these elements in the final volume, and enough of Hoagland's A–Z solution (**3**) to provide 0.01 to 0.02 p.p.m. of the minor elements were added before dilution. The reaction was finally adjusted to pH 5.8 with sulphuric acid, and maintained there by readjustment with either sulphuric acid or potassium hydroxide.

other factor must be responsible for the wilting. The presence of nitrite resulting from the hydrolysis of the urea and the subsequent incomplete oxidation of the nitrogen was suspected. The presence of a relatively high concentration of nitrite was found by qualitative test. The other urea-supplied trees which at this time showed no signs of wilting, were also tested for the presence of nitrite in the nutrient solutions, and a considerable concentration of nitrite was found in each. An attempt was made to protect these trees, as well as the wilting tree, from injury by flushing the

gravel about the roots for two days with water. The nutrient solutions were discarded and replaced with fresh solutions.

After another week, on June 15, it was noticed that all the urea-supplied trees had started to show signs of wilting, which increased in severity daily. The flushing treatment was again applied for three days. The nutrient solutions were also replaced with fresh solutions. However, the original wilting tree died after several days. The other trees recovered whenever the nutrient solutions were renewed with fresh chemicals, but severe wilting resulted after a two-day recovery period as the nitrite concentration in the solutions increased. In one case it was noted that the nitrite concentration per liter increased from zero to 42 mg. of nitrogen in a three day period (June 21 to June 24).

At no time did any of the other trees show any signs of wilting as did the urea-supplied trees. On the contrary, they continued to grow vigorously and in a healthy condition.

In order to demonstrate that the formation and increase in nitrite content of the nutrient solutions was independent of the root action or absorption by the tree, the following experiment was carried out. A urea nutrient solution was employed with and without the presence of a root system. The solutions were analyzed regularly for urea, ammonium, nitrite, and nitrate nitrogen. It was apparent (table II) that as the urea content

TABLE II

COMPARISON OF UREA NUTRIENT SOLUTIONS IN PRESENCE AND ABSENCE OF PLANTS

(THESE VALUES INDICATE MG. N. PER LITER FOR ALL FORMS OF NITROGEN)

DATE	ABSENCE OF PLANT				PRESENCE OF PLANT			
	UREA	AMMONIUM	NITRITE	NITRATE	UREA	AMMONIUM	NITRITE	NITRATE
July 9*	.....†	0	0	0	198	0	0	16
July 12	215	4	0	12	180	0	0	39
July 14	155	26	0	20	67	37	17	75
July 16	130	34	slight	28	22	58	21	83
July 19	120	66	22	22	5	64	23	78
July 21	.....†	60	25	35	5	49	16	103
July 23	85	66	28	36	.....†	41	8	100

* Fresh solutions.
† No analysis.

decreased, there was an increase in the content of ammonium, nitrite and nitrate, even in the absence of a root system. It was noted in particular that the nitrite content of the solution used in the absence of a root system reached a concentration of 28 mg. nitrogen per liter in a period of two weeks.

To demonstrate the importance of adequate aeration in preventing the accumulation of nitrite in the urea nutrient solutions, air was bubbled continuously through one urea nutrient solution, and leaving another solu-

tion unaerated as a control. The results in table III show that aeration, *i.e.*, oxygen supply, is a factor in preventing the accumulation of nitrite

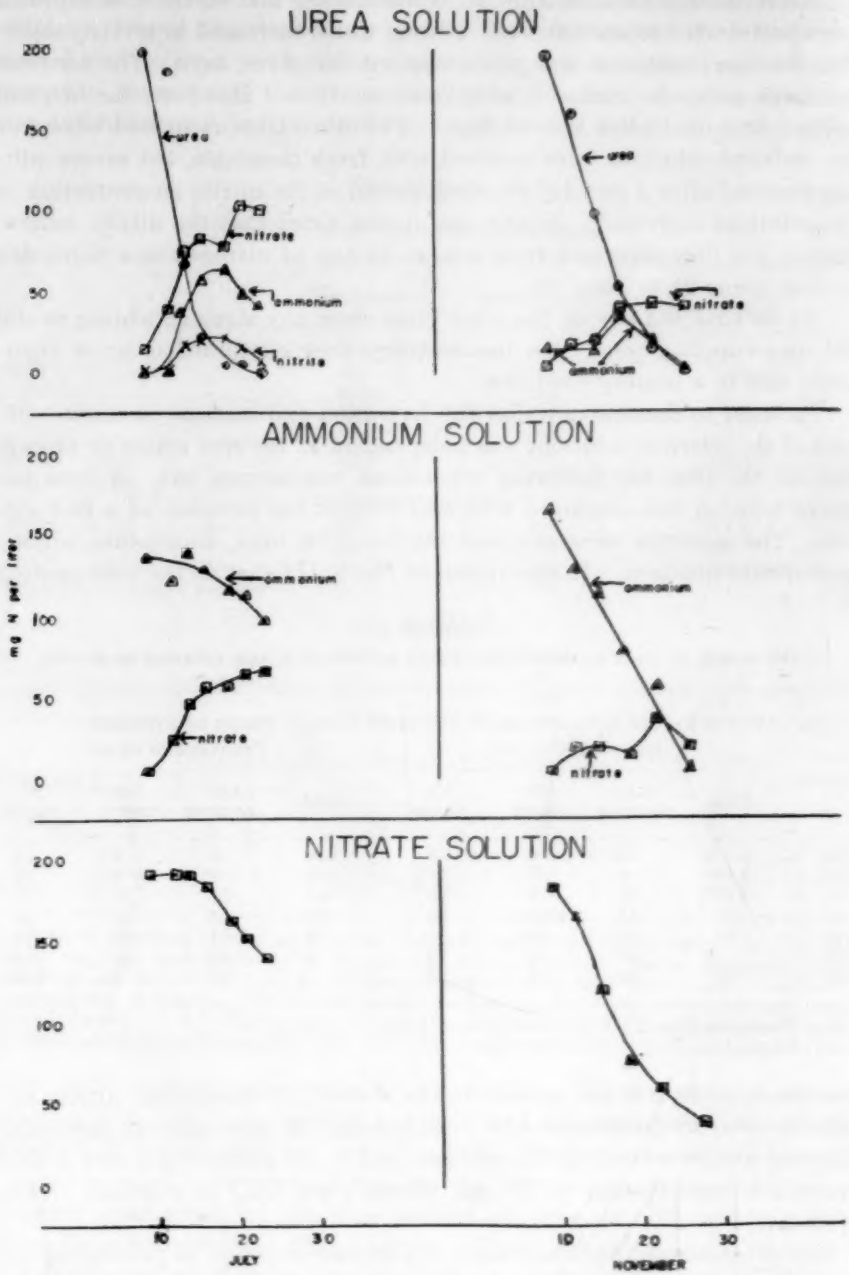

FIG. 1. Changes in the forms of nitrogen in the nutrient solutions at two different seasons of the year.

nitrogen. However, with the advent of cooler weather there was no accumulation of nitrite nitrogen in the urea nutrient solutions, even in the absence of bubbling air.

The graphs in figure 1 illustrate the changes in nitrogen in the different nutrient solutions at two different seasons of the year, and the photographs

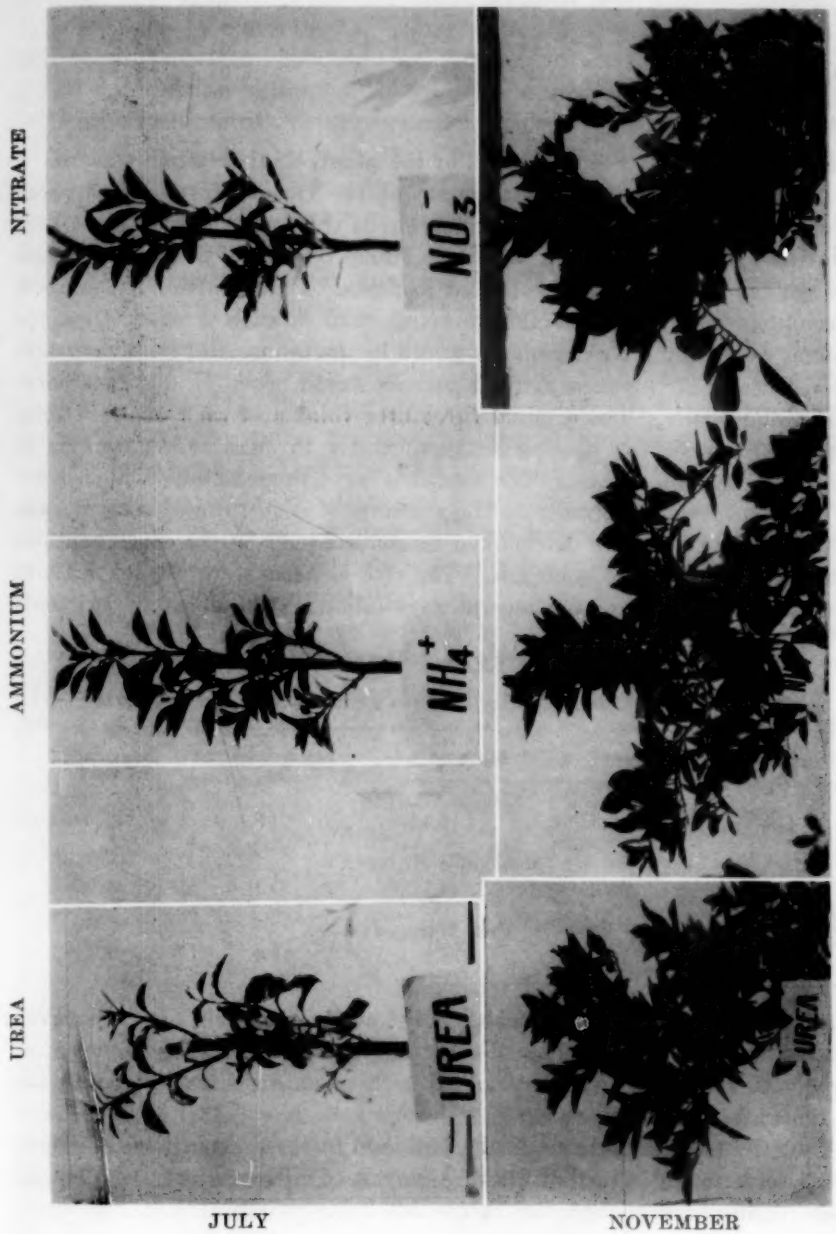

FIG. 2. Appearance of citrus trees grown in the various nutrient solutions at two different seasons of the year.

TABLE III

COMPARISON OF CONCENTRATIONS OF NITRITE NITROGEN IN AERATED AND NON-AERATED UREA NUTRIENT SOLUTIONS

	NON-AERATED	AERATED
July 12	0 mg. N. per liter	0 mg. N. per liter
July 14	26	slight
July 16	36	0
July 19	39	0
July 21	26	0
July 23	24	0

in figure 2 illustrate the conditions of the plants at these same seasons.

In order to determine the amounts of the various forms of nitrogen (urea, ammonium, and nitrate) which citrus trees may absorb, three liter quantities of each nutrient solution (table I) were prepared, passed through the containers, and collected in flasks. It took about ten minutes to complete each pass, the theory being that in such a short time the changes caused by microörganisms would be minimum, and the changes in concentrations of nitrogen in the solutions would be solely due to absorption by the plants. The original three liter volume of each nutrient solution was recovered by passing sufficient water through the containers to obtain the desired volume. The solutions were immediately analyzed for the various forms of nitrogen. The amounts of each form of nitrogen absorbed by the trees were determined by difference from the concentrations in the original nutrient solutions. The root systems were washed with 18 liter quantities of water for several days following the above experiments

TABLE IV

PER CENT. ABSORPTION OF VARIOUS FORMS OF NITROGEN BY CITRUS PLANTS

OCTOBER 6	OCTOBER 18	NOVEMBER 1	NOVEMBER 29	DECEMBER 7
		Nitrate Nitrogen		
41.3	42.3	0.3	21.8	22.0
		Ammonium Nitrogen		
20.9	38.6	44.5	29.1	22.9
		Urea Nitrogen		
----	10.6	8.2	18.4	26.5

in order to remove any nitrogen that may have been adsorbed by the gravel, and the washings were collected and analyzed for the various forms of nitrogen. These amounts were added to the quantities remaining in the solutions after the first pass.

Table IV summarizes the results obtained in these experiments. These results seem to indicate that all three forms of nitrogen are absorbed by citrus plants, even though the values are quite variable.

Discussion

The fact that the amounts of nitrite nitrogen decreased to zero, without any aeration of the urea nutrient solution, after cooler weather set in, seems to indicate that relatively high temperatures may repress the activities of the nitrite-oxidizing microörganisms which ordinarily would convert the nitrite to nitrate. While the nitrite in itself may not have been toxic, it is believed that the process of nitrate formation so depleted the oxygen supply of the nutrient solution that more economical use of the oxygen was directed to nitrite formation. According to Miller (**7**):

"Interference with the process of respiration is apparently the cause of the injury and eventual death of the roots due to the exclusion or depletion of the oxygen supply of the soil. The first visible effects of the lack of oxygen to the roots are the wilting of the plant and a lowering of the rate of transpiration. These effects are apparently due to the fact that the protoplasm does not function properly as an absorbing membrane."

It is probable that the toxic effect on the urea-supplied plants was due to a lack of sufficient oxygen about the roots, although there is also the possibility of a direct injurious effect by the high concentration of the nitrite present. CURTIS (**1**) has found that the roots of citrus seedlings were injured in nutrient cultures having an oxygen concentration of 0.7 p.p.m. or less, the injury beginning at the root tips and becoming more severe as the oxygen concentration was decreased and time of exposure to low oxygen concentration increased. CURTIS (**2**) also found that the appearance of nitrite injury on roots of citrus seedlings was not essentially different from that caused by oxygen deficiency.

The experiments cited above seem to indicate that both unfavorably high temperatures and insufficient aeration may have exerted depressing effects on the activities of the nitrite-oxidizing organisms. Since it was demonstrated that the nitrite content of the nutrient solution increased with time, it was inferred that the oxygen supply was insufficient to complete the hydrolysis of the urea and complete the oxidation of the nitrogen to the nitrate form.

$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$$

Urea

$$2NH_3 + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 2H^+$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$

Summary

Twelve trees of Valencia orange budded on rough lemon root stock were set in coarse ($\frac{1}{4}$ to $\frac{1}{8}$ inch) silica gravel. Complete nutrient solutions were used in which the form of nitrogen was varied in different solutions. The forms of nitrogen studied were urea, ammonium, and nitrate.

Wilting of trees occurred during the warmest period of the year when the source of nitrogen in the nutrient solutions was in the form of urea. Concomitant with this wilting was a very apparent accumulation of nitrite

in these solutions. Experiments demonstrated that relatively high temperatures were a factor in nitrite accumulation, which could be counteracted by sufficient aeration of the nutrient solutions.

There was also evidence that urea, ammonium, and nitrate were absorbed by the roots of citrus trees.

The authors are pleased to acknowledge their indebtedness to Dr. A. F. Camp, Vice-Director in Charge of Research, Citrus Experiment Station, Lake Alfred, Florida, for his interest in and support of this investigation.

DEPARTMENT OF AGRONOMY
UNIVERSITY OF MASSACHUSETTS
AMHERST, MASSACHUSETTS
AND
UNIVERSITY OF FLORIDA
CITRUS EXPERIMENT STATION
LAKE ALFRED, FLORIDA

LITERATURE CITED

1. CURTIS, D. S. Effect of oxygen supply in nutrient solutions on avocado and citrus seedlings. Soil Sci. **67**: 253–260. 1949.
2. CURTIS, D. S. Nitrite injury on avocado and citrus seedlings in nutrient solution. Soil Sci. **68**: 441–450. 1949.
3. HOAGLAND, D. R. and SNYDER, W. C. Nutrition of the strawberry plant under controlled conditions. Proc. Am. Soc. Hort. Sci. **30**: 288–294. 1933.
4. LEE, MARGARET H., and WIDDOWSON, E. H. The micro-determination of urea in blood and other fluids. Biochem. Journ. **31**: 2035–2036. 1937.
5. JAMIESON, V. C. The determination of nitrate, nitrite, ammonium, and urea nitrogen in sandy soils. Unpublished.
6. KIPLINGER, D. C., and LAURIE, ALEX. Growing ornamental greenhouse crops in gravel culture. Ohio Agric. Exp. Sta. Bul. 634. Oct. 1942.
7. MILLER, E. C. Plant Physiology. (2nd Ed.), p. 132. McGraw-Hill Book Co., Inc., New York. 1938.
8. NOLL, CHARLES A. Determination of nitrate in boiler water by brucine reagent. Ind. Eng. Chem. (Anal. Ed.) **17**: 426–428. 1945.
9. PEECH, MICHAEL, and ENGLISH, L. Rapid microchemical soil tests. Soil Sci. **57**: 167–195. 1944.
10. PUCHER, G. W., *et al.* Studies in the metabolism of crassulacean plants; the effect upon the composition of ***Bryophyllum calycinum*** of the form in which nitrogen is supplied. Plant Physiol. **22**: 205–227. 1947.
11. WOLF, B. Determination of nitrate, nitrite, and ammonium nitrogen. Ind. Eng. Chem. (Anal. Ed.) **16**: 446–448. 1944.
12. SNELL, F. D., and SNELL, C. T. Colorimetric methods of analysis. D. Van Nostrand Co., Inc., New York. 1936.

EFFECTS OF SOME GROWTH REGULATING SUBSTANCES ON THE TRANSPIRATION OF *ZEA MAYS*, L. AND *RICINUS COMMUNIS*, L.

MARY ANNE PLAYER

(WITH FIVE FIGURES)

Received June 9, 1949

In 1943 AMLONG (**1**) reported an increase in the transpiration of Cooper's Ernte tomato plants grown from seeds that had been soaked for 24 hours in a solution containing 100 mg. of potassium naphalene acetate, 100 mg. of ascorbic acid and 2000 mg. of thiourea per liter of water.

Three years later, BROWN (**2**) investigating the effect of 2,4-Dichlorophenoxyacetic acid (1000 p.p.m.) on the water relations of *Phaseolus vulgaris* found: "the total amount of water absorbed and transpired by sprayed plants during the five days immediately following treatment was 34% less than that for comparable untreated ones".

FERRI and AUREA LEX (**3**) added to the soil of potted plants of *Tropaeolum majus* 75 ml. of an aqueous solution of B-Napthoxyacetic acid (300 mg./1.). Twenty-four hours after treatment the transpiration rates of leaves, detached from control and treated plants, were determined. Water losses from the latter were decreased. FERRI and MERCEDES RACHID (**4**) report that 2,4-Dichlorophenoxyacetic acid (100 and 200 mg./1.) and B-Napthoxyacetic acid (various concentrations) generally caused a decrease in the transpiration rates of leaves detached from potted plants. The authors studied both monocotyledonous and dicotyledonous plants. The increasing order of effectiveness of treatment on the former was: *Coix lacryma jobi, Zea Mays* and *Stenotaphrum americanum;* of the latter: *Taraxacum officinale, Verbascum blattarioides* and *Nicotiana tabaccum.*

The results of this study, carried on during the spring (Expt. I; corn) and summer of 1947, indicate that application of the growth regulating sprays tested had a negligible effect on the water loss of corn. Contrarily, most of the sprays affected the transpiration of castor bean plants. Also, the results tend to suggest a possible correlation between the toxicity of the acid employed and the magnitude of its effect on the transpiration of the castor bean plants.

Materials and methods

Corn seeds (a single cross obtained through the generosity of Funk Bros. Seed Company) and castor bean seeds, Conner variety, (obtained through the courtesy of the United States Department of Agriculture) were used in this study. The seeds, sown in soil (three parts loam, two parts peat-moss and one part sand) in three inch pots, were allowed to grow outdoors[1] until two leaves, not cotyledons, of the plant were "mature."

[1] Corn plants were grown and Experiment I was conducted in the greenhouse.

The precaution of removing "immature" leaves and using only "mature" ones was found necessary to avoid fluctuation in leaf area during the experimental period.

Before the spray solutions were applied the pots, containing the soil and roots of the experimental plants, were completely sealed. The method described by MEYER and ANDERSON (5) was employed with one modification: an additional glass tube was inserted between the outside of the pot and the inside of the metal shell to facilitate aeration. Several times during the experimental period air was circulated in the metal shells. Hence, a lack of oxygen in the environment of the roots was not considered a factor necessitating consideration in the explanation of the results.

The weight of each plant unit: pot, shell, tubes and plant, was determined to decigrams on a trip scale. Two plants were labeled as controls. The remaining plants were divided into six groups of two plants each. Each of the six groups was then treated with one of six different growth-regulating sprays.

Indole-3-Acetic acid (IAA), B-Napthoxyacetic acid (B-NAA), P-Chlorophenoxyacetic acid (P-CAA), 2,4-Dichlorophenoxyacetic acid (2,4-D), B-(Indole-3)-Propionic acid (B-IPA), and γ-(Indole-3)-Butyric acid (γ-IBA) were prepared as aqueous solutions by dissolving the acid in 1-3 ml. of absolute ethanol and adding the appropriate amount of water. 0.01% was selected as the initial concentration for all of the sprays. In subsequent tests two other concentrations: 0.02% and 0.1% spray solutions were used. Because of the drastic epinastic effect of P-CAA and 2,4-D on dicots, the concentration of these sprays was increased only twice the original concentration. The second series of experiments with the other four acids employed 10 times as great concentrations as were used in the first tests.

Application of the spray to the plant material was, in all cases, made through the use of a small hand atomizer. Both surfaces of all blades were sprayed requiring a maximum of 10 ml. of solution per square decimeter of leaf surface.

Plants were then placed on a motor-driven table revolving three and one half to four times per minute. By means of this precaution, the environment of the plants was rendered more nearly uniform, and the complications of unequal illumination, temperature and humidity variation, or possible differences in the circulation of air around individual plant units were deemed negligible.

Plants were weighed from 8:30 to 9:30 in the evening and also between 6 and 7 in the morning in all but the first experiment involving corn, for a period of one week subsequent to the application of the sprays. Loss in weight was always replaced with the equivalent weight of water after each weighing.

In order to express results on the basis of grams of water lost per given

unit of area, the area of all leaves of both control and treated plants was determined by means of a Dietzgen's Lasico Compensating Polar Planimeter.

Results and discussion

CORN.—The magnitude of the transpiration occurring from corn plants treated with growth-regulating sprays was almost the same as that of control plants. Moreover, no epinastic responses could be observed among the plants during the week subsequent to treatment.

TABLE I

TRANSPIRATION (Gr./Dm.²) OF EACH OF THE CONTROL AND TREATED CORN PLANTS OF EXPERIMENTS I, II AND III

TREATMENT	EXPERIMENT I 18 DAYS	EXPERIMENT II 7 DAYS			EXPERIMENT III 7 DAYS		
	TOTAL	TOTAL	DAY	NIGHT	TOTAL	DAY	NIGHT
U	55.2	30.7	26.6	4.0	19.0	15.2	3.8
	40.0	31.2	27.5	3.7	18.2	13.6	4.6
C-ISF	.....	36.5	30.7	5.8	20.0	14.2	5.8
	.....	29.8	25.5	4.4	29.4	22.6	6.8
0.01% IAA	39.5	36.7	32.5	4.1	.....	.....	.....
	50.4	29.6	25.7	4.0	.....	.....	.....
0.1 % IAA	.....	.....	.....	.....	19.8	13.7	6.1
	.....	.....	.....	.....	23.2	15.9	7.3
0.01% B-NAA	49.3	35.5	31.0	4.5	.....	.....	.....
	50.5	36.9	31.8	5.1	.....	.....	.....
0.1 % B-NAA	.....	.....	.....	.....	23.5	16.0	7.5
	.....	.....	.....	.....	20.2	14.4	5.8
0.01% P-CAA	49.8	35.3	30.6	4.7	.....	.....	.....
	52.8	34.9	30.0	4.9	.....	.....	.....
0.02% P-CAA	.....	.....	.....	.....	25.1	20.9	4.2
	.....	.....	.....	.....	23.6	18.1	5.5
0.01% 2,4-D	43.9	39.2	31.5	7.7	.....	.....	.....
	52.1	33.8	28.3	5.5	.....	.....	.....
0.02% 2,4-D	.....	.....	.....	.....	18.1	14.4	3.7
	.....	.....	.....	.....	20.3	15.6	4.7
0.01% B-IPA	46.8	28.7	25.1	3.6	.....	.....	.....
	38.8	31.2	26.7	4.6	.....	.....	.....
0.1 % B-IPA	.....	.....	.....	.....	22.4	16.2	6.1
	.....	.....	.....	.....	25.0	18.1	6.9
0.01% γ-IBA	57.9	34.1	29.0	5.1	.....	.....	.....
	45.5	22.9	19.8	3.1	.....	.....	.....
0.1 % γ-IBA	.....	.....	.....	.....	22.3	15.6	6.8
	.....	.....	.....	.....	26.5	17.8	8.6

A summary of three experiments is presented in table I, which gives the total water loss (Gr./Dm.²) of each plant, treatment, and the length of the experiment. Average daily water loss, shown cumulatively, is depicted in figures 1, 2, and 3.

In the second and third experiments Ivory Soap Flakes were added to each of the six sprays (0.5 gm./100 cc.) to insure a more nearly even dis-

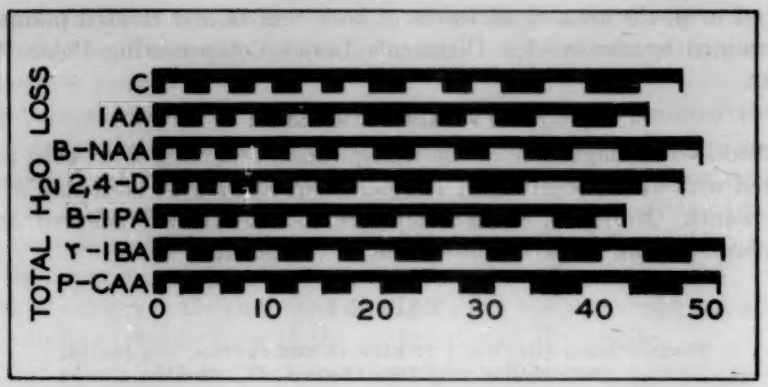

FIG. 1. Average water losses of corn plants shown cumulatively for 18 days subsequent to treatment with sprays. Each segment of the horizontal bars represents a successive 24 hour period. Treatment on the ordinate (concentration of each acid: 0.01%). Transpiration in Gr./Dm.^{2} on the abscissa.

tribution of the growth regulating substances over the surfaces of the pubescent corn blades. Plants designated C-ISF were, of course, treated with an aqueous solution of Ivory Soap Flakes (0.5 gm./100 cc.). U indicates untreated plants.

From table I (Experiment I) it may be seen that the difference in the total amount of water lost by the two control plants (15.2 gm.) is almost

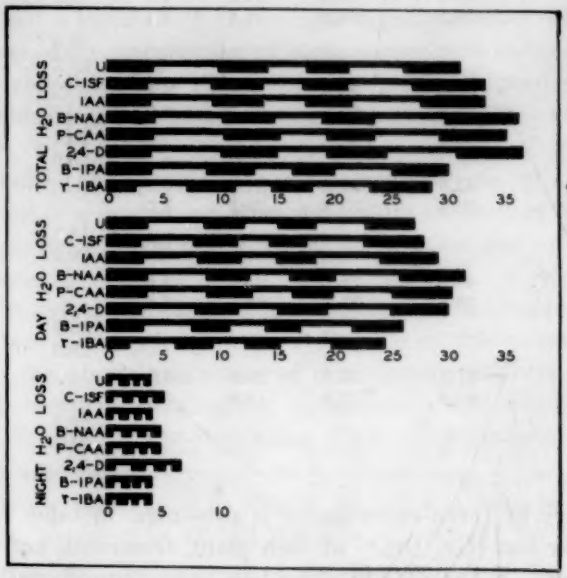

FIG. 2. Transpiration (Gr./Dm.^{2}) of corn plants shown cumulatively for a week subsequent to the application of 0.01% sprays. Each segment of all average total water loss bars represents a successive 24 hour period; day loss a 13 hour period; night loss an 11 hour period.

as great as the difference between any treated plant and the controls. Moreover, the differences obtained by subtracting the average total water losses of the sprayed plants from the average total water loss of the control plants does not exceed 5 grams: approximately one-third the variation in total water loss of the two control plants. Therefore, it seems valid to assume that the sprays had little or no effect on the transpiration of the treated plants. Experiments II and III reaffirm the negligible effect of the growth regulating substances on the transpiration of corn.

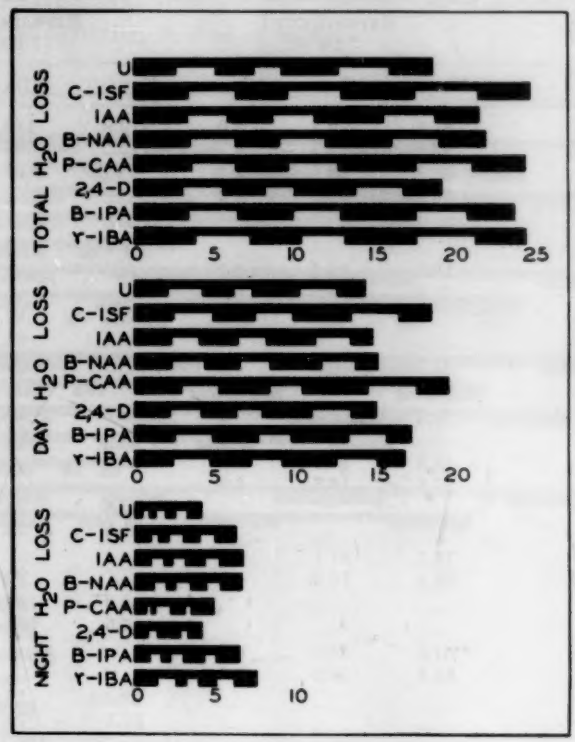

FIG. 3. Transpiration (Gr./Dm.^{2}) of corn plants shown cumulatively following treatment (concentration of P-CAA and 2,4-D was 0.02%; of other sprays 0.1%). Each segment of an average total water loss bar represents 24 hours; segments of day loss: 13 hours; of night loss: 11 hours.

CASTOR BEANS.—The most general effect of the various growth-regulating sprays on the transpiration of castor beans was a decrease in water loss which varied with the spray as well as its concentration.

Within an hour after treatment plants showed varying degrees of epinasty. These responses generally became more severe during the next two or three days, and for plants sprayed with P-CAA and 2,4-D wilting of the leaves and death of the plants by the end of the week was observed in both experiments.

A summary of two experiments is given in table II. In both experiments the differences between the average total water losses of each of the groups of treated plants, except plants treated with B-IPA, and the average total water loss of the control plants is greater than the variation in the amount of water lost by the control plants in the experiment.

TABLE II.

TRANSPIRATION (Gr./Dm.^{2}) OF EACH OF THE CONTROL AND TREATED CASTOR BEAN PLANTS

TREATMENT	EXPERIMENT I 7 DAYS			EXPERIMENT II 7 DAYS		
	TOTAL	DAY	NIGHT	TOTAL	DAY	NIGHT
C	81.5	76.5	5.0	119.9	113.8	6.1
	78.0	73.3	4.7	112.2	106.8	5.4
0.01% IAA	61.2	56.5	4.7	.....	.....	.....
	66.3	61.0	5.3	.....	.....	.....
0.1 % IAA	.....	.....	.....	68.5	61.9	6.6
	.....	.....	.....	61.7	55.1	6.6
0.01% B-NAA	78.0	71.9	6.1	.....	.....	.....
	71.2	66.2	5.1	.....	.....	.....
0.1 % B-NAA	.....	.....	.....	94.4	82.4	12.0
	.....	.....	.....	90.5	81.2	9.3
0.01% P-CAA	48.0	40.8	7.2	.....	.....	.....
	64.6	55.6	9.0	.....	.....	.....
0.02% P-CAA	.....	.....	.....	89.2	76.2	13.0
	.....	.....	.....	78.9	65.7	13.2
0.01% 2,4-D	25.3	20.5	4.8	.....	.....	.....
	20.8	16.8	4.0	.....	.....	.....
0.02% 2,4-D	.....	.....	.....	30.5	24.1	6.4
	.....	.....	.....	31.6	24.1	7.5
0.01% B-IPA	73.5	67.1	6.4	.....	.....	.....
	81.3	76.3	4.9	.....	.....	.....
0.1 % B-IPA	.....	.....	.....	108.0	100.6	7.4
	.....	.....	.....	115.5	108.3	7.2
0.01% γ-IBA	61.3	55.3	6.0	.....	.....	.....
	64.9	59.2	5.6	.....	.....	.....
0.1 % γ-IBA	.....	.....	.....	83.6	76.5	7.1
	.....	.....	.....	86.1	79.1	7.0

Since the obvious decreases in transpiration of plants sprayed with IAA, B-NAA, P-CAA, 2,4-D, and γ-IBA are consistently accentuated by increased concentrations of these respective acids it seems valid to conclude that all of the sprays tested, except B-IPA, are effective in decreasing the total transpiration of castor bean plants. Moreover, it also seems justifiable to conclude that the decrease in water loss occurs during the hours of daylight (figures 4 and 5).

A most singular result to be noted however, is an increased transpiration during the night hours of castor bean plants treated wiht 2,4-D, B-NAA and P-CAA. The compounds are increasingly effective, in the order given, in promoting this effect. Increased concentrations of each of these sprays intensifies the results.

UNIVERSITY OF MICHIGAN LIBRARIES

A possible explanation of this phenomena might be the increased surface area exposed by splitting of the stems of plants sprayed with 2,4-D, P-CAA and sometimes IAA. B-NAA, however, is not especially active in promoting, at least macroscopically, the splitting of stems of castor bean plants.

The explanation of a deviation in the total amount of water lost by two plants subjected to identical treatment is thought to be due, not only to the difference in the degree of splitting of stem tissue, but also to unequal nastic movements of the leaves of two plants. It is believed that partial overlapping and protection of some of the transpiring areas may in part account for the variation in loss of water.

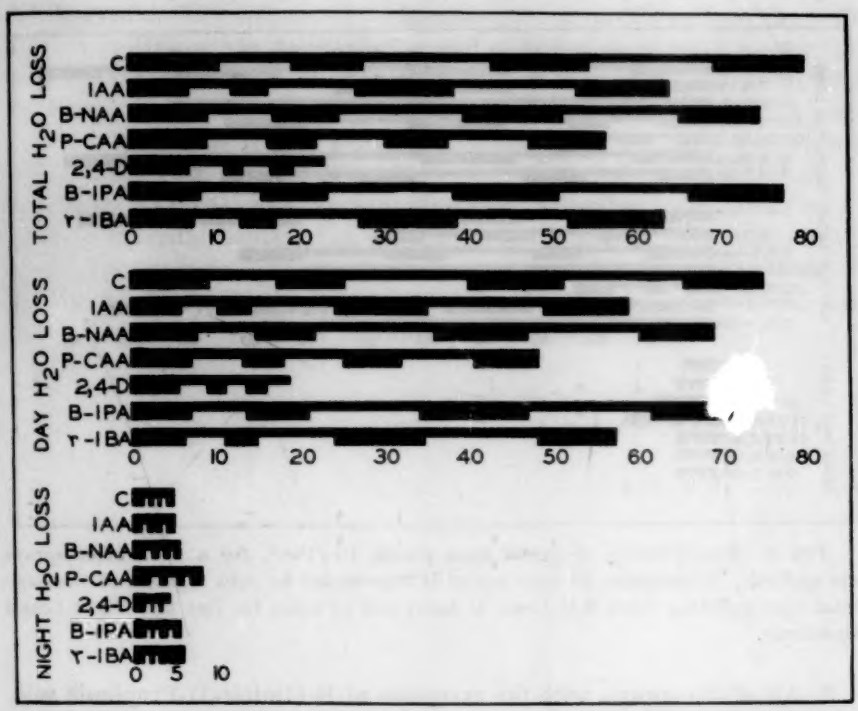

FIG. 4. Transpiration (Gr./Dm.^{2}) of castor bean plants shown for a week subsequent to the application of 0.01% acids. Each segment of the horizontal bars representing a successive 24 hour period (Total H_2O Loss); 13 hours (Day) or 11 hours (Night Loss).

This reasoning is thought to be applicable, for example, to the 16.6 grams difference between the total water loss in grams per square decimeter of the two plants sprayed with 0.01% P-CAA (table II); also to the 10.3 grams difference between those plants sprayed with 0.02% P-CAA. However, in the latter instance the variation is not of much greater magnitude than the deviation: 7.7 grams, between the two controls for that experiment.

Summary

Six growth-regulating compounds: Indole-3-Acetic acid, B-Napthoxyacetic acid, P-Chlorophenoxyacetic acid, 2,4-Dichlorophenoxyacetic acid, B-(Indole-3)-Propionic acid and γ-(Indole-3)-Butyric acid were tested in the following concentrations: P-Chlorophenoxyacetic acid and 2,4-Dichlorophenoxyacetic acid at 0.01% or 0.02%; the other compounds at 0.01% or 0.1% concentrations to determine their effects on the transpiration of *Zea Mays*, L. and *Ricinus communis*, L., (variety: Conner).

1. Average total transpiration of treated corn plants (Gr./Dm.^{2}) was not considered to be affected by treatment with the growth-regulating sprays.

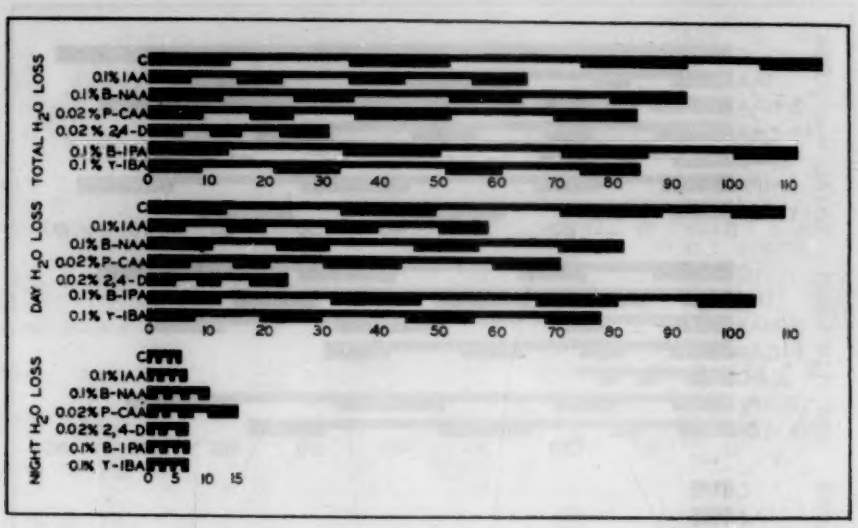

FIG. 5. Transpiration of castor bean plants, Gr./Dm.^{2}, for a week after sprays were applied. A successive 24 hour period is represented by each segment of the horizontal bars depicting Total H_2O Loss; 13 hours and 11 hours for Day and Night Losses respectively.

2. All of the sprays, with the exception of B-(Indole-3)-Propionic acid, decreased the average total transpiration of castor bean plants. Also, an increase in the concentration of each of the effective sprays resulted in a greater decrease in the average total water loss of the treated plants. The order of effectiveness of the sprays in decreasing transpiration of castor bean plants was consistently: 2,4-Dichlorophenoxyacetic acid, P-Chlorophenoxyacetic acid, Indole-3-Acetic acid, γ-(Indole-3)-Butyric acid and B-Napthoxyacetic acid.

3. Increase in the average total transpiration of treated castor bean plants during the hours of the night, in comparison with the average total transpiration of comparable untreated plants was repeatedly observed. A possible theory is proposed for the explanation of this observation.

The author wishes to express sincere appreciation to Dr. R. O. Freeland, at whose suggestion this problem was undertaken, for his advice and counsel during the course of the research. Also to thank her father, Mr. H. P. Player for innumerable hours of assistance as experimentation was in progress.

DEPARTMENT OF BOTANY
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS

LITERATURE CITED

1. AMLONG, H. U. Uber den Einfluss der Hormonisierung auf die Transpiration der Pflanze. Naturwiss. **31**: 44–45. 1943.
2. BROWN, J. W. Effects of 2,4-Dichlorophenoxyacetic acid on the water relations, the accumulation and distribution of solid matter, and the respiration of bean plants. Bot. Gaz. **107**: 332–343. 1946.
3. FERRI, MARIO G., and LEX, AUREA. Stomatal behavior as influenced by treatment with B-Napthoxyacetic acid. Contr. Boyce Thompson Inst. **15**: 283–290. 1948.
4. FERRI, MARIO G., and RACHID, MERCEDES. Further information on the stomatal behavior as influenced by treatment with hormone-like substances. Anais. Acad. Bras. Cienc. **21**: 155–166. 1949.
5. MEYER, B. S., and ANDERSON, D. B. Laboratory Plant Physiology. page 28. Edwards Brothers, Inc. Michigan. 1940.

RESPIRATION STUDIES ON CHLORELLA. I. GROWTH EXPERIMENTS WITH ACID INTERMEDIATES

DÉSIRÉ M. ENY[1]

(WITH ONE FIGURE)

Received April 30, 1949

Introduction

The study of carbohydrate metabolism has received considerable attention in the animal domain. It has been assumed by many that carbohydrate metabolism in higher plants follows a similar path. Many investigators have contributed toward the elucidation of this assumption but the final goal has not yet been reached. The literature on the subject has been ably surveyed by VICKERY *et al.* (**19**), PUCHER *et al.* (**11**), and SIDERIS *et al.* (**16**) and will not be reviewed in detail in the present paper.

The reactions occurring during the metabolism of carbohydrates are separated into two main schemes—one taking place under anaerobic, the other under aerobic conditions. The problems having received the most attention are largely concerned with anaerobic processes such as the fermentation of sugar to ethyl alcohol and the formation of lactic acid. The reactions leading to the complete oxidation of carbohydrates under aerobic conditions (*i.e.* respiration) are not as well understood.

The object of the present investigation is to study the possible steps involved in the respiratory mechanism of plant tissues inasmuch as they are connected with the aerobic path of carbohydrates, by determining the extent to which some of the metabolic intermediates known for animal tissues fit into the plant domain.

The program of research was planned to include several parts, the first being the study of the growth of chlorella when supplied with organic acids known to enter in the path of aerobic carbohydrate metabolism in animal tissues. Other portions are concerned with the effects of organic acids on respiration and the study of the action of various enzymes and enzyme inhibitors. These will be reported in subsequent publications.

Materials and methods

MATERIALS.—The strain of chlorella used was originally isolated from soil by WANN (**20**) and has been maintained in pure culture in the Plant Physiology laboratory at Cornell University. According to CLARK (**2**), the species has not been described taxonomically.

The chlorella used were all derived from a single cell obtained by plating out a suspension of cells at the beginning of the investigation. The algae

[1] Present address: Firestone Plantations Company, Harbel, Liberia, West Africa.

were grown in the nutrient solution used by FLEISHER (3) and by MANDELS (8), with the addition of Haas and Reed's A–Z solution. The full nutrient solution is composed of:

KNO_3	1.25 gm.	.0124 M
$MgSO_4 \cdot 7H_2O$	2.46 gm.	.0100 M
KH_2PO_4	1.22 gm.	.0090 M
$FeCl_3$	.01 gm.	
Redistilled H_2O	1000 ml.	
A–Z solution	1 ml.	
Na citrate	1.00 gm.	.0014 M
Glucose	15.00 gm.	

Throughout this study the term "nutrient solution" refers to the full nutrient solution described above but minus the Na citrate and glucose.

All the chemicals and reagents used were of C.P. grade. Triple distilled water was used throughout for solutions, rinsing glassware, etc. The purity of this triple distilled water, when measured with a Barnstead purity meter, registered about .05 part per million of NaCl, whereas the ordinary distilled water contained .8 p.p.m. of which .1 p.p.m. is known to be copper. The .05 p.p.m. of impurities of the triple distilled water can be accounted for by the uptake of CO_2 from the air.

When used, the organic acids were added to a concentrated nutrient solution and then made to volume so as to keep the final concentration of the nutrient solution intact.

The acid intermediates used were:

Lactic	Succinic
Pyruvic	Fumaric
Acetic	Malic
Cis-Aconitic	Butyric
Citric	Propionic

Unfortunately oxalacetic and isocitric acids were not available. α-ketoglutaric acid was obtained in a quantity insufficient to utilize for the growth experiments.

The acids were used in the salt form. When a salt was not commercially available, it was either synthesized, as in the case of pyruvate by Robertson's procedure (14), or the acid was neutralized with NaOH and brought to the needed concentration. Cis-aconitic acid was prepared from commercial trans-aconitic acid by the Malachowski and Maslowski method (7).

By means of a Beckman automatic pH meter (sensitivity = ± .1), the initial pH of the solutions was regulated with a few drops of normal HCl or NaOH to about 5.6, which was found to be favorable for the growth of chlorella. This operation, however, was omitted when glucose was used because glucose lowered the pH about .2.

PREPARATION OF CULTURES.—The stock cultures were grown in 1 liter Erlenmeyer flasks, each of which contained about 300 ml. of the full nutrient solution while the organic acid culture vessels were 300 ml. Erlenmeyer flasks, each containing 100 ml. of nutrient solution. The flasks were sterilized by autoclaving at 15 pounds of pressure for 20 minutes.

Stock culture vessels were inoculated with a transfer loop and the organic acid cultures were inoculated with pipettes. The pipettes used for inoculation were sterilized in a hot air oven at 160° C to two hours. A small wad of cotton was inserted in the mouth end before sterilization.

The flasks were then placed in the insulated thermostated chamber as described by MANDELS (8) and were automatically shaken for five minutes every hour. The light intensity applied for stock cultures was about 500 foot candles. On the other hand, the organic acid cultures were kept in the dark. For this purpose, the insulated chamber was protected from light by four layers of black cotton cloth. In both cases, the temperature of the chamber was adjusted to 28° C ± .5°.

In preparing the inoculum for organic acid cultures, a known amount of cells was needed to permit an accurate measurement of growth. To free them of sugar, the cells were removed from full nutrient solution and starved for 30 hours. As freedom from other organisms was essential, the centrifuge could not easily be used. Consequently all operations were made in the transfer room which had been sprayed with a dilute solution of mercuric bichloride and the working surfaces wiped with a dilute solution of phenol. The cells were allowed to settle in the stock flasks. The supernatant liquid was discarded and about 200 ml. of sterilized nutrient solution were poured in. The flasks were shaken thoroughly and the contents allowed to settle. This operation was repeated six times at one hour intervals, after which about 100 ml. of nutrient solution were added. The resulting suspension was allowed to stand in the dark at room temperature for 30 hours. One ml. of this suspension was inoculated into each flask containing the organic acid cultures. This stock suspension, if kept in the refrigerator for periods up to 21 days, will give equally good results. However, in all the experiments reported here, no stock suspension older than 12 days was used.

CELL COUNTS.—Cell density was determined with a haemocytometer of the type used for blood counts. Samples were taken with an inoculating loop.

CHLOROPHYLL DETERMINATION.—A chlorophyll extraction procedure similar to that of FLEISCHER (3) was used. An aliquot of cell suspension was centrifuged, the supernatant liquid discarded and enough distilled water to cover the cells was added. The centrifuge tubes were heated in a boiling water bath for two minutes. The suspension was centrifuged again and the supernatant discarded. The pigments were extracted by adding two or three 15 ml. amounts of methyl alcohol and then boiling the mix-

ture for two minutes. Two or three small bits of carborundum were placed into each tube to prevent bumping during the boiling process. The extracts were combined and brought to a final volume of 50 ml.

To determine the amount of chlorophyll, it was first necessary to obtain a solution containing a known concentration of chlorophyll and then to construct a calibration curve for the electric photometer which was used in subsequent routine determinations.

The COMAR and ZSCHEILE (1) method was followed to obtain the solution of known concentration. This process requires the use of the Beckman spectrophotometer which gives the optical density $\left(D = \log_{10} \frac{I_o}{I}\right)$ of the sample. The values found are then substituted in the equation:

$$\text{Total chlorophyll mg./ml.} = 7.12 \log_{10} \frac{I_o}{I} \text{ (at 6600 Å)} + 16.8 \log_{10} \frac{I_o}{I} \text{ (at 6425 Å)}.$$

For the routine determinations, a Fisher electric photometer was used with the micro attachments and the red filter (6500 Å).

DETERMINATION OF ACIDS.—The nutrient solutions containing acid were tested to determine the amounts of acid that may have been withdrawn by the chlorella which had grown in it for varied periods. The quantities were titrated potentiometrically by the Van Slyke and Palmer method (18). This method is based on the theory that if the salt of a weak acid is present in a water solution, the addition of nearly one full molecule of HCl for each molecule of such salt is necessary to cause a change in the hydrogen ion concentrations from 10^{-8} to 2×10^{-3}. However, if the electrolytes present are salts of strong acids such as sulphites or chlorides, relatively little strong mineral acid is required to change the pH.

The organic acids to be determined all behave as weak acids. As the nutrient solutions also contain weak mineral acids, notably phosphoric and carbonic which would interfere with the determination, these mineral acids must be eliminated before titration. A 10 ml. aliquot of the suspension was centrifuged, the supernatant liquid poured into a beaker, the cells washed with distilled water and the washings added to the beaker after centrifugation. A small amount of powdered $Ca(OH)_2$ was added to the beaker to precipitate the mineral acids which were then left to stand for 15 minutes and stirred occasionally. The suspension was filtered and the filtrate brought to pH 7.8 with two normal NaOH. This was titrated potentiometrically with the Beckman pH meter to pH 2.6. A distilled water blank was deducted from the final titration and a correction factor was applied to obtain the titration value which was converted to milliequivalents per milliliter of suspension.

The correction factor was calculated from the percentage of recovery of

the acids obtained on known amounts of the individual acids. The recovery per cents. are given below.

Acid	Recovery per cent.
Lactic	93.5
Pyruvic	68.5
Acetic	99.2
Cis-aconitic	80.6
Citric	90.0
Succinic	98.5
Fumaric	83.5
Malic	93.5
Butyric	98.7
Propionic	95.3

Results

During the normal process of reproduction, one chlorella parent cell usually produces two to four autospores which, after enlarging, rupture the parent cell wall and are liberated, growing thereafter as individual cells. Growth curves of chlorella are similar to those of bacterial populations and, during the period of active cell division, the increase in cell number is an exponential function. Plotting the logarithm of the number of cells versus time gives a straight line. However, where cell division is slow, this type of plotting offers no special advantage.

In the present studies, it was necessary to remove all sources of carbohydrates except those to be tested. Consequently the major portion of the experiments was conducted in the dark to eliminate the photosynthetic production of carbohydrates. As a result, the growth was much slower than for chlorella grown in light or with glucose added to the nutrient solution. For this reason, the logarithmic plotting was not used. Instead, growth was measured by counting the number of cells per $mm.^3$ of suspension.

The concentration of chlorophyll was determined to give an indication of the health conditions of the cells. To compare the condition of the chlorella grown in organic acid cultures with that of the chlorella grown in glucose, the chlorophyll concentration of cells grown with glucose in the dark was potted against the number of cells in the suspension as a reference curve. Figure 1 shows the straight line relationship obtained. It denotes that the nutrient solution was suitable for chlorella since the chlorophyll content is proportional to the number of cells. There is, however, a large margin of variation for cells grown with other organic nutrients.

The various sodium salts of organic acids added to the nutrient solutions were used at many different concentrations. However, only those concentrations giving the most significant results have been reported in the ensuing data. The acids are listed, not alphabetically, but logically, in

the order in which they are generally described in the tricarboxylic cycle of animal tissues.

SODIUM LACTATE.—Lactate has been suggested by several investigators as a source of organic matter which could replace glucose in many cultures. WARBURG (21) measured the respiration of chlorella which had been fed lactic acid in the light. He found a much lower rate of oxygen uptake than when the algae were grown with glucose. No comparative growth was reported however.

In this study, table I shows that growth occurred with lactate at several levels of concentrations. As little as .005 M gave a ratio of growth of 1.63 when the relative growth of the control was 1.0. Even a concen-

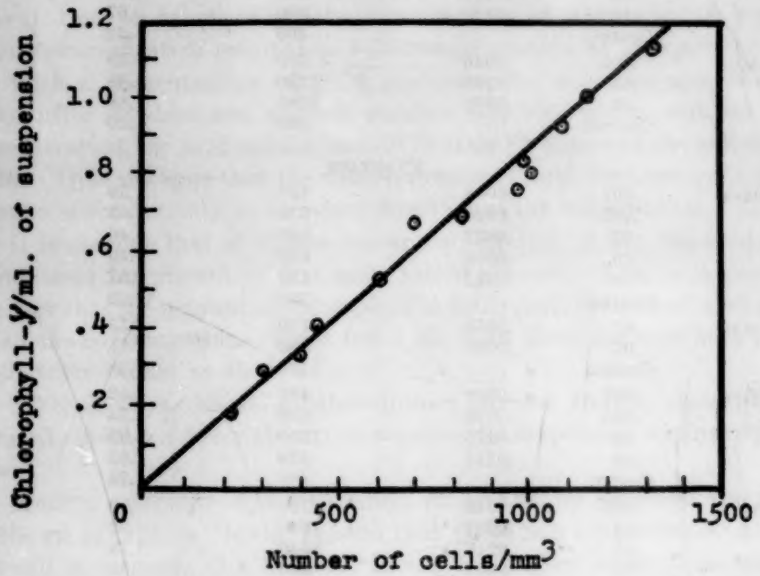

FIG. 1. Relationship between the chlorophyll content and the concentration of cells in suspension.

tration of .0005 indicated a greater cell number than the control. The best growth, however, occurred with .1 M lactate, which gave a ratio of 4.0 35 days after inoculation. Above .1 M growth decreased.

Throughout the period of growth, the amount of acid present in the cultures decreased. Column 3 of table I shows the amount of acid disappearance. Larger amounts of acid were lost from the nutrient solutions which originally contained the highest concentrations. A correlation between the amount of acid absorbed and the increase in the number of cells cannot be expected since other life processes might be involved which would utilize acids without resulting in growth. However, the fact that cell growth was better when more acid was absorbed is a good indication that lactic acid is assimilated by chlorella.

TABLE I

Duration	Concentration of acid at start Me./ml.*	Organic acids absorbed Me./ml.*	Number of cells per mm.³ of suspension	Chlorophyll content γ/ml. of suspension	Growth ratio**
			Lactate		
8 Days	.005	.0012	360	.28	1.00
	.01	.0030	410	.26	1.13
	.05	.0035	520	.39	1.45
	.10	.0047	560	.40	1.56
	Control		360	.28	
15 Days	.05	.0064	560	.38	1.47
	.10	.0072	640	.44	1.68
	Control		380	.28	
23 Days	.005	.0042	440	.35	1.10
	.05	.0157	960	.71	2.40
	.10	.0126	880	.67	2.20
	Control		400	.32	
35 Days	.005	.0046	560	.39	1.63
	.05	.0240	1250	.88	3.91
	.10	.0302	1280	.88	4.00
	Control		380	.20	
			Pyruvate		
8 Days	.001	.0008	420	.30	1.11
	.005	.0026	530	.41	1.36
	.01	.0032	580	.46	1.49
	.05	.0046	640	.49	1.64
	.10	.0012	450	.37	1.15
	Control		390	.29	
16 Days	.01	.0058	720	.55	1.80
	.05	.0090	880	.71	2.20
	Control		400	.28	
24 Days	.001	.0009	480	.32	1.20
	.005	.0048	680	.53	1.70
	.05	.0173	1250	.95	3.13
	.10	.0115	820	.62	2.05
	Control		400	.28	
38 Days	.005	.0049	750	.59	1.88
	.01	.0097	1240	.92	3.10
	.05	.0248	1640	1.18	4.10
	.10	.0208	950	.59	2.38
	Control		400	.26	
			Acetate		
8 Days	.001	.0009	540	.41	1.38
	.01	.0037	560	.40	1.44
	.05	.0054	620	.47	1.59
	.10	.0024	580	.44	1.49
	Control		390	.29	
16 Days	.05	.0114	630	.47	1.66
	.10	.0045	570	.41	1.50
	Control		380	.26	
25 Days	.001	.00096	530	.39	1.26
	.05	.0101	960	.81	2.29
	.10	.0135	710	.56	1.69
	Control		420	.29	
36 Days	.01	.0099	680	.52	1.89
	.05	.0304	1550	1.09	3.97
	.10	.0167	780	.61	2.17
	Control		360	.22	

* Me./ml. = milliequivalents per milliliter.

** Growth ratio = $\frac{\text{number of cells in the nutrient solution + acid.}}{\text{number of cells in plain nutrient solution}}$

Growth ratio of the control = 1.0.

UNIVERSITY OF MICHIGAN LIBRARIES

The chlorophyll concentration increased with the number of cells, but, as the growth period was lengthened, some variations occurred, indicating a definite tendency toward lower chlorophyll content.

At first the increase in cell number was very slow. After the 15th day, the rate of cell division increased, but after the 35th day a yellowing of the cells was observed and no increase in cell number occurred. After about 50 days, the cells were colorless and clumped. As this phenomenon occurred with all the acids tried, it will be enlarged upon at the end of this section.

SODIUM PYRUVATE.—After 38 days in .05 M pyruvate, the cell number increased from 400 to 1640 cells per $mm.^3$ which represents a growth ratio of 4.1 (table I). This growth ratio was not improved by increasing the growth time or by changing the concentration of pyruvate. A lower or higher concentration resulted in a decreased number of cells per $mm.^3$

With a concentration of .1 M pyruvate, the acid disappearance was .0208 after 38 days and the cell number was 950, while, with an .05 M concentration, the acid uptake was .0173 after 24 days and the cell number 1250. Thus it seems that the disappearance of acid does not vary in proportion with, but only in the same direction as the cell number.

It is possible that at higher concentrations (*i.e.*, .1 M), the acid intake is not used for growth or that some toxicity occurs. This is indicated by the fact that the amount of chlorophyll is fairly well correlated with growth in all the concentrations except for .1 M, when there are only 59 γ per ml. while there should be about 84 γ.

After 38 days, the cell number did not increase and the chlorophyll decreased rapidly. After about two months, the suspension was nearly colorless.

SODIUM ACETATE.—The utilization of acetate by chlorella for growth is shown in table I. It can be seen that .05 M is a concentration at which growth is optimum at all times. Lower and higher levels of acetate gave poorer growth. The chlorophyll content did not denote good correlation with the concentration of cells. Even for the maximum growth, the amount of chlorophyll was appreciably lower than normal (fig. 1). It should also be noted that a disappearance of acid from the culture solution occurred. This disappearance is closely related to growth.

Between 26 to 28 days after inoculation, yellowing of the cells was apparent and after 36 days, no more growth was observed. After about 46 days, the cells were colorless and clumped.

SODIUM ACONITATE.—The results for aconitate, shown in table II, indicate that aconitate is utilized for growth. Chlorella was best adapted to the aconitate concentration of .04 M which gave a growth ratio of 2.97 after 35 days. Above .08 M, the rate of growth decreased and, at approximately .4 M, growth completely ceased. The chlorophyll content of the cells did not increase proportionately with the increase of cells. For in-

stance, there were 51 γ of chlorophyll for 640 cells after 25 days in .04 M aconitate and only 50 γ of chlorophyll for 660 cells after 35 days in .02 M aconitate.

Column 3 shows that acid disappearance is more highly correlated with the concentration of acid originally present in the suspension than with the amount of growth, although best growth occurred when more acid had disappeared. For instance, the best growth, 1160 cells, was obtained when the acid disappearance was at a maximum .0326. It is likely that a large part of the acid is either broken down through respiration or by some other processes rather than being utilized for food storage and growth.

SODIUM CITRATE.—The maximum growth obtained when using citrate was 660 cells with a concentration of .02 M after 28 days (table II). This corresponded to a growth ratio of only 1.65 despite the fact that the acid disappearance from the nutrient solution was as high as .0225 me./ml. As the growth ratio with citrate was consistently above 1, it must be concluded that citrate can be utilized by chlorella.

It is important to note that the chlorophyll content did not increase in relation to cell development after 15 days of growth and that after 20 days, cell development occurred with even some loss of chlorophyll. After 28 days, cell counts remained constant but chlorophyll decreased and disappeared. At about 40 days, the cells formed a colorless mass.

SODIUM SUCCINATE.—The data on the growth of chlorella obtained with succinate is presented in table II. The best growth, which was 700 cells/mm.^{3}, occurred for a concentration of .02 M after 30 days. At that time the ratio of growth for the concentrations listed varied from 1.29 to 1.67. This indicates that nearly as much growth was obtained for smaller concentrations of acid as when larger amounts were used. For example, .0212 me./ml. gave a ratio of 1.67 as compared with a ratio of 1.29 for .0092 me./ml. More than twice the amount of acid disappeared in the first case as in the latter.

Similarly the chlorophyll obtained for .0092 me./ml. of acid disappearance was 39 γ and only 53 γ for .0212 me./ml. of acid disappearance. Consequently, although succinate was used for growth, it was not utilized efficiently.

After 30 days, growth was very sparse and after 45 days, the cells formed a filamentous mass with signs of disintegration.

SODIUM FUMARATE.—Seven days after inoculation, the number of cells reached 650/mm.^{3} in .01 M fumarate and only 630/mm.^{3} in .04 M (table III). However, after 33 days, the concentration in .01 M reached 840 cells while .04 M fumarate gave 1100, its highest cell concentration.

The amount of acid disappearance for the culture solution varied in the same direction as growth, but it must be observed that only a very small amount of acid disappeared for the highest fumarate concentration used (.0068 me./ml. for .08 M concentration after 33 days). This seems

TABLE II

Duration	Concentration of acid at start Me./ml.*	Organic acids absorbed Me./ml.*	Number of cells per mm.³ of suspension	Chlorophyll content γ/ml. of suspension	Growth ratio**
			Aconitate		
8 Days	.06	.0062	410	.33	1.14
	.12	.0076	530	.40	1.47
	.24	.0085	560	.42	1.56
	Control		360	.28	
16 Days	.12	.0094	580	.43	1.53
	.24	.0126	600	.45	1.58
	Control		380	.26	
25 Days	.12	.0147	640	.51	1.68
	.24	.0158	620	.47	1.63
	Control		380	.25	
35 Days	.015	.0082	490	.39	1.26
	.06	.0164	660	.50	1.69
	.12	.0326	1160	.81	2.97
	.24	.0287	890	.68	2.28
	Control		390	.24	
			Citrate		
8 Days	.015	.0043	380	.30	1.00
	.06	.0057	540	.43	1.42
	.15	.0072	430	.37	1.13
	.30	.0066	480	.39	1.26
	Control		380	.29	
15 Days	.06	.0115	600	.45	1.58
	.15	.0142	560	.42	1.47
	Control		380	.29	
20 Days	.015	.0052	400	.26	1.00
	.06	.0186	660	.45	1.65
	.15	.0153	560	.41	1.40
	.30	.0074	480	.31	1.20
	Control		400	.29	
28 Days	.06	.0225	660	.40	1.65
	.15	.0183	540	.43	1.35
	Control		400	.27	
			Succinate		
8 Days	.01	.0038	460	.37	1.07
	.04	.0046	480	.38	1.12
	.10	.0062	520	.42	1.21
	.20	.0046	450	.39	1.05
	Control		430	.36	
15 Days	.10	.0085	540	.41	1.29
	.20	.0074	480	.36	1.14
	Control		430	.36	
22 Days	.04	.0124	620	.46	1.48
	.10	.0116	560	.44	1.33
	.20	.0164	520	.39	1.24
	Control		420	.30	
30 Days	.01	.0092	540	.39	1.29
	.04	.0212	700	.53	1.67
	.10	.0234	600	.44	1.43
	.20	.0263	580	.44	1.38
	Control		420	.29	

* Me./ml. = milliequivalents per milliliter.

** Growth ratio = $\frac{\text{number of cells in the nutrient solution + acid.}}{\text{number of cells in plain nutrient solution}}$

Growth ratio of the control = 1.0.

TABLE III

Duration	Concentration of acid at start Me./ml.	Organic acids absorbed Me./ml.	Number of cells per mm.³ of suspension	Chlorophyll content γ/ml. of suspension	Growth ratio**
			Fumarate		
7 Days	.02	.0046	650	.49	1.55
	.08	.0040	630	.47	1.50
	.16	.0015	480	.38	1.14
	Control		420	.32	
14 Days	.08	.0072	770	.59	1.83
	.16	.0037	580	.46	1.38
	Control		420	.34	
22 Days	.08	.0234	960	.85	2.29
	.16	.0056	660	.55	1.57
	Control		420	.31	
33 Days	.02	.0127	840	.65	1.95
	.08	.0365	1100	.83	2.56
	.16	.0068	670	.50	1.56
	Control		430	.34	
			Malate		
7 Days	.10	.0052	650	.50	1.59
	.20	.0020	460	.36	1.12
	Control		410	.28	
14 Days	.10	.0082	750	.58	1.74
	.20	.0041	570	.44	1.33
	Control		430	.34	
24 Days	.01	.0072	680	.49	1.62
	.10	.0246	920	.72	2.19
	.20	.0106	700	.54	1.67
	Control		420	.30	
33 Days	.01	.0092	830	.63	1.89
	.10	.0276	980	.75	2.23
	.20	.0135	680	.51	1.55
	Control		440	.34	
			Butyrate		
8 Days	.05	.0024	480	.37	1.26
	.10	.0019	420	.34	1.11
	Control		380	.28	
15 Days	.05	.0043	540	.43	1.38
	.10	.0025	480	.36	1.23
	Control		390	.28	
25 Days	.05	.0087	590	.43	1.56
	.10	.0080	490	.38	1.29
	Control		380	.26	
35 Days	.005	.0037	460	.36	1.21
	.05	.0121	780	.61	2.05
	.10	.0102	560	.41	1.47
	Control		380	.25	
			Propionate		
8 Days	.05	.0020	460	.36	1.15
	.10	.0018	410	.35	1.03
	Control		410	.35	
15 Days	.05	.0046	500	.40	1.22
	.10	.0044	480	.38	1.17
	Control		410	.32	
25 Days	.05	.0076	580	.42	1.38
	.10	.0068	510	.41	1.21
	Control		420	.32	
35 Days	.005	.0044	490	.34	1.17
	.05	.0115	660	.52	1.57
	.10	.0112	580	.46	1.38
	Control		420	.32	

UNIVERSITY OF MICHIGAN LIBRARIES

to indicate that at this and higher concentrations, fumarate is not taken up by the cells.

The amount of chlorophyll in the cells was fairly well correlated with the concentration of cells and also with the amount of acid disappearance.

As in the case of previously mentioned acids, after a period of about 33 days, growth ceased, yellowing of the cells was observed and at about 48 to 50 days, the suspensions became colorless.

SODIUM MALATE.—Table III gives the results obtained with malate. The best growth was obtained with a concentration of .05 M after a period of 33 days. The growth ratio at that concentration was 2.23. It is interesting to see that a malate concentration of .1 M gave a poorer growth than the lower concentration of .005 M. In fact after 24 days in .1 M, the growth was slightly better than at 33 days which is an indication that these cells become deficient in some substance essential for growth more rapidly than those in a lower concentration of malate or that some sort of toxicity sets in as the cells did not stop growing because of a lack of organic acid. This is further brought out by the variations in chlorophyll content. After 24 days, the chlorophyll decreased for the cells in .1 M and increased slightly in the cells grown in .05 M concentration. After 28 days, yellowing was noticed in all the cultures and at about two months, most of the cells were discolored. No more growth was measured after 33 days.

It can be observed that less acid was used when there were 830 cells than at a cell concentration of 680. This may indicate that more acid was utilized in the second case for metabolic processes other than growth, as for instance, respiration.

SODIUM BUTYRATE.—Butyrate is not an intermediate of the aerobic cycle in animal tissues. It represents, however, one of the first links of fat metabolism with carbohydrate metabolism (**13**). MYERS (**10**) mentioned he had obtained some evidence that butyric acid was respired by chlorella. Hence, the possibility that butyrate might be assimilated for growth was signified and experiments were conducted with butyrate as with the organic acids listed.

The results obtained for butyrate are shown in table III. Of all the concentrations used, .05 M consistently gave the best growth. Thirty-five days after inoculation, the cell number reached a maximum of 780/mm.^{3} which represents a growth ratio of 2.05. The amount of acid disappearance was then .0121 me./ml. The cell count was 560/mm.^{3} for cells grown in .1 butyrate. Hence at the highest concentration of butyrate shown, chlorella did not make very efficient use of the acid which it may have consumed in excessive respiration or other maintenance life processes.

The chlorophyll content of the cells was generally low but followed a somewhat uniform trend. Yellowing of the cells occurred between 33 and 40 days starting in the culture having the highest concentration of butyrate.

Growth ceased at about the same time and discoloration was complete after two months.

SODIUM PROPIONATE.—Propionate is not an intermediate of the aerobic cycle in animal tissues. Like butyrate, however, it is closely related to it through fat metabolism.

Results obtained by adding propionate to the nutrient solution are reported in table III. The maximum growth ratio was 1.57 after 35 days in .05 M. Changing the concentration between .005 M and .1 M only resulted in a variation of growth of 1.17 to 1.57. However fairly large amounts of propionate, up to .0115 me./ml. disappeared from the nutrient solution. It is thus indicated that very little propionate was used for growth. As propionate is often used as a mold inhibitor in the preservation of dairy products, it is possible that the low growth obtained in this case is also due to toxicity.

Growth which occurred until about 35 days ceased after that time. The chlorophyll content of the cells which was somewhat below normal decreased rapidly after 35 days. After 43 days, the cells were nearly colorless and clumped.

USE OF ACID MIXTURES.—The growth obtained with the various acids was poor when compared to the growth obtained with glucose. To cover the possibility that several acids together might give a better growth, many mixtures of acids were tried with a number of varied concentrations. The mixtures giving the most significant results are reported in table IV.

TABLE IV

GROWTH OF CHLORELLA WITH ACID MIXTURES

MIXTURE NUMBER	COMPOSITIONS OF THE MIXTURES	NUMBER* OF CELLS
1	.02 M Lactate + .02 M Pyruvate + .02 M Acetate + .01 M Aconitate + .01 M Succinate + .01 M Fumarate + .01 M Butyrate	1530
2	.02 M Pyruvate + .02 M Acetate + .01 M Aconitate + .01 M Fumarate	1860
3	.02 M Pyruvate + .02 M Aconitate + .02 M Citrate	760
4	.02 M Pyruvate + .02 M Citrate + .02 M Malate	750
5	.02 M Pyruvate + .02 M Succinate	710
6	.02 M Aconitate + .003 M Citrate + .02 M Succinate + .003 M Fumarate + .005 M Malate	480
7	.003 M Aconitate + .005 M Citrate + .005 M Succinate + .02 M Fumarate + .01 M Malate	680
8	.01 M Aconitate + .01 M Citrate + .01 M Succinate + .01 M Fumarate + .01 M Malate	550

* Maximum number of cells per mm.³ of suspension obtained for each mixture.

Maximum growths obtained in periods varying from 30 to 38 days are given here. Except for mixtures 1 and 2, which gave 1530 and 1860 cells respectively, all the mixtures containing pyruvate gave poorer results than pyruvate alone, which gave 1640 cells. Mixture number 2 was the only mixture giving better growth than .05 M pyruvate. It is interesting to observe that

when citrate or succinate were present, growth was decreased, except for mixture number 1 containing .01 M succinate which gave a comparatively good growth.

Although the acid mixtures did not give the expected growth results, they are at least indicative of the relative value of the acids when used together.

Discussion

The relative value of the individual acids is further reviewed in table V

TABLE V

SUMMARY OF GROWTH RESULTS

ORGANIC ACID	MOLAR CONCENTRATION	CELL* NUMBER	GROWTH RATIO	ORDER** NUMBER
Lactate	0.10	1280	4.00	3
Pyruvate	0.05	1640	4.10	1
Acetate	0.05	1550	3.97	2
Aconitate	0.04	1160	2.97	4
Citrate	0.02	660	1.65	8
Succinate	0.02	700	1.67	9
Fumarate	0.04	1100	2.56	5
Malate	0.05	980	2.23	6
Butyrate	0.05	780	2.05	7
Propionate	0.05	660	1.57	10
(Glucose)	0.08	34500	88.46	.....
(Control)	.....	380	1.00	.....

* Number of cells per mm.^{3} of suspension.

** Order in which the acids gave the highest growth results after the maximum growth period.

which shows the concentration of acid giving the best growth and also the maximum cell concentration obtained for each acid.

Lactate was best utilized by chlorella at a concentration of .1 M. All the other acids used gave best results at concentrations of .05 M or lower. Citrate and succinate gave the poorest growth although both of these acids were removed from the solutions in fairly large quantities. It was shown that the addition of citrate or succinate to the acid mixtures (table IV) had the effect of reducing growth. It is possible that these acids accumulate in chlorella and produce a toxic effect.

There is still argument as to whether or not citrate lies in the main path of the aerobic cycle in animal tissues. KREBS (**4**) who first suggested this cycle has very recently presented further evidence in favor of it (**5**). However WERKMAN and WOOD (**22**) and other investigators have given data to the effect that citrate is the product of a side reaction of aconitate.

The present results show that citrate is not utilized as rapidly as the other acids by chlorella and would therefore favor the second viewpoint. Normally citrate may be present in chlorella only in a very minute quantity and the amount of aconitase present may be too small to take care of a citrate accumulation.

In the case of succinate, however, it is difficult to speculate as to what is the cause of this low growth. Succinate is an intermediate in the aerobic cycle of animal tissues. If the cycle were similar in plants, succinic acid should normally give the same effect as fumarate or malate. The results obtained here may signify that, in chlorella, succinic acid does not follow the path indicated in the tricarboxylic cycle. This would mean that the succinic acid step is by-passed and that another mechanism is available for the formation of some of the four-carbon acids. Succinic itself would be only a side product resulting from the condensation of two pyruvates or two acetates, as occurs in bacteria. In that case, the explanation of the succinic results would be similar to that given for citric acid.

It has been shown that after a period varying from 30 to 45 days, depending upon the acid used, cell division stopped in all the cultures. Tests for acid remaining in the nutrient solutions, made at different times between 30 and 50 days, show that all solutions of more than .01 M at the start still had a fairly large amount of acid left and that the acid continued to decrease but at a slower rate. After the death of the cells, some esterification of the acid or other chemical changes may take place which would account for the decrease of acid in the solution. This esterification could also occur before growth ceased due to the death of cells even while growth is occurring.

It was observed that the decrease in chlorophyll content was noticeable at least a few days before growth stopped. Whether this is an early indication occurring due to the cessation of growth or whether it was a cause of it was not determined. That the time at which chlorella stopped growing is similar for all the acids would indicate that some essential factors are being used which are not resynthesized in the dark when acids are the sole source of energy, although they are in the presence of glucose. As glucose has an energy level much higher than that of the acids used, this may be a plausible explanation. The growth of plants without chlorophyll would eliminate chlorophyll as a primary factor.

It is also possible that some elements become unavailable through changes in pH. The pH were tested on several occasions but the changes were not significant enough to prevent the growth of chlorella as chlorella has a fairly large range of pH. The growth ratio with glucose was 88.46 as compared with 4.10, the highest ratio reached with any of the acids (pyruvate). However, all ratios were above 1. It can be concluded that although the organic acids used are extremely poor materials for chlorella growth, they are all assimilated in various degrees in the probable order given in table V.

The reversibility of the cycle of metabolism in plant tissues has been a very debated subject. That plants are capable of converting certain organic acids into carbohydrates has been mentioned on several occasions in the literature. For instance, in 1905, TREBOUX (17) found that the growth

of chlorella in light was better with butyric or with lactic acid than with the control containing no acid. LWOFF and DUSI (6) found that acetate was a satisfactory source of carbon for the growth in the dark of some species of Flagellata. According to RABINOWITCH, (12) SABALITCHKA and WEIDLING (15) found that *Elodea canadensis* also can form starch from acetaldehyde in the dark.

In the present experiments, the fact that definite growth is due to the presence of the organic acids indicates that they are used by chlorella as a source of carbon. As the acids are also functional in the aerobic path of carbohydrate metabolism in animal tissues, it is justifiable to suggest that the aerobic path of carbohydrate metabolism in chlorella is similar along certain broad lines to that of animals. Obviously since the death of chlorella occurred after a limited time, an additional source of energy which may be supplied in the form of glucose or exposure to light, is necessary for indefinite proliferation.

Fat accumulation in chlorella has been noted by several investigators (9). The assimilation of butyrate and propionate also indicates that a fat metabolism connected with the aerobic carbohydrate cycle takes place.

Summary

Organic acids, known to exist as intermediates in the path of carbohydrate metabolism of most animals and bacteria, were supplied in the dark to the green alga, chlorella and its growth was studied with a view toward elucidating the respiratory mechanism.

The acids included lactic, pyruvic, acetic, cis-aconitic, citric, succinic, fumaric and malic. Acids not listed above but occurring in the aerobic cycle of carbohydrate metabolism were not available. Butyric and propionic acids which are involved in fat metabolism were also used in these experiments.

Growth continued for a limited length of time but was much slower than that supported by glucose. It was affected by the particular acid used and by the concentration.

The approximate relative order of assimilation of the acids by chlorella, the concentration of each acid producing the highest number of cells, and the order of magnitude (ratio) of growth have been reported.

As the taxonomically unclassified species of chlorella studied was able to utilize for growth without light the organic acids tested, it may then be inferred that chlorella can build reduced compounds such as carbohydrates, proteins and fats for a limited time without a photochemical reaction.

The author wishes to thank Professor Lewis Knudson for his helpful advice and suggestions during this investigation.

DEPARTMENT OF BOTANY
CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

1. Comar, C. L., and Zscheile, F. P. Analysis of plant extracts for chlorophylls a and b by a photoelectric spectrophotometric method. Plant Physiol. **17**: 198–209. 1942.
2. Clark, D. G. Cornell Univ. Personal communication. 1948.
3. Fleischer, W. E. The relation between chlorophyll content and rate of photosynthesis. J. Gen. Physiol. **18**: 573–597. 1935.
4. Krebs, H. A. The role of citric acid in intermediate metabolism in animal tissues. Enzymologia **4**: 148–156. 1937.
5. Krebs, H. A. The tricarboxylic acid cycle. Harvey lecture series. New York Academy of Medicine. March 1949.
6. Lwoff, A., and Dusi, M. La nutrition azotée et carbonée de *Chlorogonium euchlorum* á l'obscurité; l'acide acétique envisagé comme produit de l'assimilation chlorophylienne. Compt. Rend. Soc. Biol. **119**: 1260. 1935.
7. Malachowski, R., and Maslowski, M. Ber. 61. B: 2521. 1928, in Umbreit, W. W., Burris, R. H., and Stauffer, J. F. Manometric Techniques. Burgess Pub. Co., Minneapolis, Minnesota. 1947.
8. Mandels, G. R. A quantitative study of chlorosis in chlorella under conditions of sulphur deficiency. Plant Physiol. **18**: 449–462. 1943.
9. Meyers, J. Culture conditions and the development of the photosynthetic mechanism. III. Influence of light intensity on cellular characteristics of chlorella. J. Gen. Physiol. **29**: 419–427. 1946.
10. Meyers, T. Oxidative assimilation in relation to photosynthesis in chlorella. J. Gen. Physiol. **30**: 217–227. 1947.
11. Pucher, G. W., Leavenworth, C. S., Ginter, W. D., and Vickery, H. B. Studies in the metabolism of crassulacean plants: The effects of temperature upon the culture of excised leaves of *Bryophyllum calycinum*. Plant Physiol. **23**: 123–132. 1948.
12. Rabinowitch, E. L. Photosynthesis. Vol. I. Interscience Pub., New York. 1945.
13. Rittenberg, D., and Bloch, K. The utilization of acetic acid for fatty acid synthesis. J. Biol. Chem. **154**: 311–312. 1944.
14. Robertson, W. B. The preparation of sodium pyruvate. Science **96**: 93–94. 1942.
15. Sabalitchka, T. and Weidling, H. Biochem. Z. **172**: 210. 1926.
16. Sideris, C. P., Young, H. Y., and Chun, H. H. Q. Diurnal changes and growth rates as associated with ascorbic acid, titratable acidity, carbohydrate and nitrogenous fractions in the leaves of *Ananas comosus* (L.) Merr. Plant Physiol. **23**: 38–69. 1948.
17. Treboux, O. Einige Stoffliche Einflüsse auf die Kohlensaure Assimilation bei Submerzen Pflanzen. Flora. **92**: 49–76. 1903.

UNIVERSITY OF MICHIGAN LIBRARIES

18. VAN SLYKE, D. D., and PALMER, W. W. Studies of acidosis. XVI. The titration of organic acids in urine. J. Biol. Chem. **41**: 567–585. 1920.
19. VICKERY, H. B., PUCHER, G. W., WAKEMAN, A. J., and LEAVENWORTH, C. S. Chemical investigations of the tobacco plant. VI. Chemical changes that occur in leaves during culture in light and in darkness. Connecticut Agr. Expt. Sta. Bull. 399. 1937.
20. WANN, F. B. The fixation of free nitrogen by green plants. Amer. Jour. Bot. **8**: 1. 1921.
21. WARBURG, O. Biochem. Z. **177**: 471. 1926.
22. WERKMAN, C. H., and WOOD, H. G. On the metabolism of bacteria. Bot. Rev. **8**: 1–68. 1942.

MINERAL COMPOSITION OF CHLOROTIC ORANGE LEAVES AND SOME OBSERVATIONS ON THE RELATION OF SAMPLE PREPARATION TECHNIQUE TO THE INTERPRETATION OF RESULTS

PAUL F. SMITH, WALTER REUTHER, AND ALSTON W. SPECHT

Received March 16, 1950

Certain types of chlorosis of leaves of green plants are universally attributed to deficiencies or unbalanced conditions of one or more of the essential mineral elements. Agreement does not always exist, however, as to whether a specific type of leaf-symptom pattern is related to total content of the deficient element or only to a specific fraction of it. This is particularly true of the presumed deficiencies of iron and zinc where sample selection, contamination, and poor precision of analytical procedures are all complicating factors. An extensive review of all previous work is beyond the scope of this report. JACOBSON (**8**) and LINDNER and HARLEY (**10**) point out some of the more pertinent discrepancies with respect to iron content and iron chlorosis. More recently MCGEORGE (**11**) reports the lack of any apparent relationship between total Fe or Zn and the appearance of the specific deficiency symptoms for these elements in citrus, pear, and several other plants. Other studies have occasionally shown this same result in the case of Fe; but JACOBSON (**8**) found that surface contamination was a factor of major importance in producing discrepant results. When pear leaves from trees grown in calcareous soil were carefully washed in dilute HCl, a correlation was found between the low total Fe values found and the iron chlorosis shown. BENNETT (**1**) confirmed this observation with further studies, although he found a stronger relationship of chlorosis to an acid-soluble, or "active" Fe fraction than to the total. In Florida (**6**), New Zealand (**7**), and elsewhere citrus trees showing typical zinc deficiency symptoms have been found to contain less total zinc in their leaves than leaves from healthy trees.

In Florida when the acidity of the topsoil of the Lakeland and related series drops below pH 5.0 (between 4.5 and 5.0) for a considerable length of time, citrus trees may become unthrifty in appearance, sparsely foliated, and show typical iron-chlorosis leaf patterns. This interveinal chlorosis condition strongly resembles the lime-induced iron chlorosis pattern (**2**) and that associated with poor drainage. Exploratory tests in small containers showed that reducing the acidity by treatment with the carbonates of Ca, Na, or K (or extracting the soil with NH_4-acetate) all resulted in the disappearance of this symptom in young citrus seedlings. In connection with this general problem, some analyses were made to determine the mineral composition of leaves from trees affected with this type of chloro-

UNIVERSITY OF MICHIGAN LIBRARIES

sis. This led to preliminary tests on the preparation of leaf samples for analysis. Several methods of washing were compared, and these were found to influence the apparent concentration of some of the elements present in small amounts. Total iron was found to be consistently lower in chlorotic leaves than in green leaves when both were carefully cleansed.

This report is concerned with the mineral composition of leaves from orange trees affected with this particular type of acid-soil chlorosis, and it gives certain information about sample preparation.

Materials and methods

Preliminary tests showed that, at least for the macro-nutrient elements, only minor variations in composition existed among the several leaves produced on a twig when a flush of growth occurred. This finding afforded an easy means of preparing several samples of whole leaves having almost identical composition. A number of twigs were harvested and samples were prepared in such a way that each twig contributed one leaf to each sample. By selecting twigs with leaves of uniform size, 6 to 8 reasonably identical samples were prepared for comparison of different washing treatments.

The washing treatments used were: (1) Wiped with moistened cheesecloth, (2) scrubbed with a fiber-bristled hand-brush in tap water, (3) scrubbed in 0.3N HCl, (4) scrubbed in a detergent[1] solution for different lengths of time, and (5) scrubbed in a detergent solution that was acidulated to 0.3N with HCl. All washing solutions were made with tap water. Each sample was rinsed in running tap water and in four separate distilled water baths. The drying, grinding (in a semi-micro Wiley mill equipped with a nickel-coated screen), storage, and analysis were all carried out as previously described (**13, 14**). Iron determinations made by standard chemical procedures on the 32 samples summarized in table II agreed closely with the spectrographic values listed.

In order to ascertain the amount of contamination contributed by the use of the Wiley mill, six samples were crushed manually with a porcelain mortar and pestle. These samples are compared with matched samples that were ground in the mill. One set of samples was collected from trees that had been sprayed with Cu and Zn to give some measure of the efficacy with which these elements could be removed. Four samples of picked leaves were sprayed with a ferrous sulphate solution containing hydroxylamine and Dreft and allowed to dry for 30 minutes before washing. This spray mixture showed some greening response when sprayed on chlorotic leaves on the trees.

[1] Dreft, a commercial "soapless" washing powder (sodium lauryl sulphate) was the only detergent tried. CHAPMAN *et al.* (4) suggest using Ivory soap, and BENNETT (1) states that a "detergent" is efficacious in removing dust residues from pear leaves.

Results and discussion

Iron was assumed to be a general surface contaminant and was used as an indicator of efficacy of washing in initial tests. Since nearly all citrus leaves have on them visible dust particles, no samples were analyzed without some washing treatment. One of the initial tests was to take twigs of growth from a mature seedling orange tree that appeared to be "normal" and form samples as described above. Duplicate samples were scrubbed with tap water, in 0.3N HCl, and in Dreft suds. The following total iron values were found respectively: 125, 155; 61.4, 62.8; and 47.5, 46.9 p.p.m. The detergent wash seemed somewhat superior to the acid wash and both were effective in greatly lowering the apparent iron concentration in the leaves. Since these values were lower than most of the published values for healthy leaves of tree crops, it was felt worthwhile to repeat the test on leaves that showed the iron-chlorosis pattern, taken from trees on very acid soil. In addition to the three treatments just described, one sample was wiped carefully with water-moistened cheesecloth, and two additional periods of detergent washing were included. The same strength of detergent wash was used for all three periods. One set of severely chlorotic, one set of mildly chlorotic, and two sets of green samples were taken from trees all in a small area of an orchard (Hamlin orange on Rough lemon roots). The leaves were mature when collected in December 1946. Table I shows the results, which are not entirely consistent. Scrubbing in water seems to have removed a larger proportion of the surface contamination from green leaves than from chlorotic ones. The acid wash was not superior to water alone on the green leaves, but appeared to be so on the chlorotic leaves. The detergent-washed leaves tended to become slightly lower in iron content with prolonged washing; but did not differ from each other significantly. This would indicate that the leaching out of internal leaf-iron was probably not a factor in these tests. The correspondence between the degree of greenness and total Fe content of the detergent-washed samples is striking.

Another collection of leaves from the same orchard was made during the rainy season of 1947. About 4-month-old leaves were collected on June 16. Two samples of mildly chlorotic leaves and two samples of green, each with 15 leaves, were given the washing treatments indicated in table I, with two added treatments, one in which it was desired to see if freshly applied iron could be removed and one in which the detergent was acidulated with HCl. The samples were analyzed for 11 mineral elements (table II). The similarity in composition of samples matched in the manner described is apparent. The only elements affected by the washing treatments were Fe, Al, Cu, and Na. There was apparently very little external matter on these leaves, which was probably due to the washing of the rains. The lowest Fe values were again found with the detergent-washed leaves. The behavior of Al, in respect to washing treatment, was

UNIVERSITY OF MICHIGAN LIBRARIES

identical to that of Fe. Copper likewise was lower in the leaves that were washed with the detergent, although some slight irregularities exist in the green-leaf series. Sodium and Fe apparently entered the leaves of the sprayed groups, as they averaged substantially higher for both of these elements. Likewise Na was found to increase with increase in time of detergent washing in the case of chlorotic leaves (the detergent contains Na). Green leaves were not significantly affected in this respect. The chlorotic leaves were significantly higher than the green leaves in N, P, K, and Na and lower in Ca, Mg, Fe, Mn, Cu, and B. Both types of leaves showed the same Al content.

A comparison of the proportion of each of these elements found in the leaves of a healthy Pineapple orange orchard on the same soil type, with the pH maintained above 5.0, can be seen in table III. The samples involved in this case were collected about three weeks later in the same year

TABLE I

THE EFFECT OF DIFFERENT METHODS OF WASHING ON THE APPARENT IRON CONTENT* OF HAMLIN ORANGE LEAVES

CONDITION OF LEAVES	WIPED WITH CHEESE-CLOTH	H_2O 3 MIN.**	0.3N HCl 3 MIN.	DREFT			MEAN
				1 MIN.	3 MIN.	6 MIN.	
Severely chlorotic	68.5	44.6	26.2	13.6	12.8	10.7	29.4
Mildly chlorotic	66.3	59.4	35.6	36.0	35.0	33.8	44.4†
Green A	125.1	51.6	51.2	44.9	42.1	40.2	59.2††
Green B	87.5	53.9	51.7	52.4	49.0	44.2	56.4††
Mean	86.8	51.9††	41.2††	36.5††	34.7††	32.2††	

* P.p.m. Fe on oven-dry basis (50° C).

** Entire sample in contact with washing medium for three minutes. During this time each leaf was scrubbed on both surfaces with a small wooden, fiber-bristled hand-brush.

† Difference from first mean is significant at odds greater than 19: 1.

†† Difference from first mean is significant at odds greater than 99: 1.

as those in table II. The most striking differences are the low Fe and Mn values found in the leaves from the orchard showing chlorosis. Again detergent washing resulted in lower Fe and Cu values than did the acid washing.

One washing test was made on leaves from old sweet seedling trees that were showing partial recovery from the acid-soil injury. Lime application had been made several months previous to sampling, and the chlorosis pattern was difficult to find. A few weeks before sampling the orchard was sprayed with a mixture containing $CuSO_4$, $ZnSO_4$, and hydrated lime. The leaves collected showed a very faint chlorotic pattern with the veins slightly greener than the interveinal areas. Triplicate samples were treated as shown in table IV. Zinc was determined in addition to the other trace elements already mentioned.

It is apparent that grinding these samples in the mechanical mill had

UNIVERSITY OF MICHIGAN LIBRARIES

TABLE II

THE EFFECT OF SEVERAL METHODS OF WASHING HAMLIN ORANGE LEAVES ON THE MINERAL CONTENT FOUND BY ANALYSIS

WASHING TREATMENT	PERCENTAGE IN DRY LEAVES					P.P. M. IN DRY LEAVES					
	N	P	K	Ca	Mg	Fe	Mn	Cu	B	Na	Al
Leaves showing moderate "iron" chlorosis pattern											
Wiped with moist cheesecloth	3.11	0.175	2.77	2.50	0.313	27.8	30.6	9.7	74.1	499	37
Scrubbed in tap water (3 min.)*	3.06	0.172	2.88	2.38	0.303	31.3	27.9	9.7	75.9	509	35
Scrubbed in 0.3N HCl (3 min.)	3.00	0.175	2.70	2.30	0.311	30.2	27.4	9.4	74.8	486	35
Scrubbed in Dreft (1 min.)	3.07	0.176	2.78	2.45	0.303	24.7	29.3	8.5	75.9	515	28
Scrubbed in Dreft (3 min.)	3.00	0.172	2.73	2.53	0.317	23.1	27.3	7.8	74.5	536	21
Scrubbed in Dreft (6 min.)	2.98	0.173	2.74	2.62	0.331	21.4	27.1	8.4	74.5	629	18
Scrubbed in Dreft + 0.3N HCl	3.00	0.171	2.67	2.53	0.311	24.1	28.4	8.0	74.3	561	21
Scrubbed in Dreft (3 min.)**	2.99	0.173	2.68	2.50	0.300	62.1	28.0	8.2	78.2	776	22
Mean for chlorotic-type leaves	3.026	0.1734	2.744	2.476	0.3111	30.59	28.25	8.71	75.28	563.8	27.12
Green leaves from trees adjacent to those above											
Wiped with moist cheesecloth	2.80	0.147	2.16	3.03	0.358	39.3	37.0	10.6	83.6	433	34
Scrubbed in tap water (3 min.)	2.79	0.154	2.08	3.00	0.348	36.3	35.6	13.0	81.8	431	28
Scrubbed in 0.3N HCl (3 min.)	2.79	0.159	2.08	3.00	0.348	37.5	37.3	12.6	83.5	418	34
Scrubbed in Dreft (1 min.)	2.85	0.155	2.18	2.91	0.346	34.4	35.0	11.8	81.6	396	24
Scrubbed in Dreft (3 min.)	2.78	0.147	2.17	2.93	0.361	35.0	39.2	10.6	83.3	432	24
Scrubbed in Dreft (6 min.)	2.84	0.148	2.18	3.02	0.360	33.9	38.3	10.7	82.8	441	18
Scrubbed in Dreft + 0.3N HCl	2.94	0.155	2.16	2.90	0.348	33.7	38.4	10.0	78.5	434	21
Scrubbed in Dreft (3 min.)**	2.80	0.154	2.09	2.99	0.356	54.8	36.7	10.3	81.2	514	20
Mean for green-type leaves	2.824	0.1524	2.138	2.972	0.3531	38.11	37.19	11.20	82.04	437.4	25.38
L.S.D.† between leaf-type means 0.05	0.61	0.004	0.13	0.07	0.007	1.58	1.62	0.44	2.62	39	N.S.
L.S.D. between leaf-type means 0.01	0.83	0.006	0.18	0.09	0.009	2.17	2.21	0.59	3.56	53	N.S.
L.S.D. between any two values 0.05	N.S.	N.S.††	N.S.	N.S.	N.S.	2.24	N.S.	0.62	N.S.	55	4.65
within leaf-type 0.01	N.S.	N.S.	N.S.	N.S.	N.S.	3.07	N.S.	0.84	N.S.	74	6.22

* Length of time that the leaves were in contact with the washing solution.

** Leaves sprayed with 0.25% $FeSO_4 \cdot 7H_2O$ + 0.25% $NH_2OH \cdot HCl$ + 0.5% Dreft and allowed to dry 30 min. before washing.

† L.S.D.—Least significant difference at level indicated.

†† N.S.—Not significant.

TABLE III

EFFECT OF DREFT AND HCl AS WASHING AGENTS ON THE MICRO-NUTRIENT CONTENT OF 24 MATCHED PAIRS OF SAMPLES OF HEALTHY PINEAPPLE ORANGE LEAVES

SAMPLE TREATMENT	PERCENTAGE IN DRY LEAVES					P.P.M. IN DRY LEAVES				
	N	P	K	Ca	Mg	Fe	Mn	Cu	B	Na
Scrubbed in Dreft	2.65	0.141	2.11	3.01	0.426	90.69	86.72	6.92	77.34	527.2
Scrubbed in 0.3N HCl	.....	.....	.....	.....	.....	93.53	88.05	7.17	78.02	530.4
Effect of treatment	.....	.....	.....	.....	.....	**	N.S.	*	N.S.	N.S.

* Odds for significant difference between means exceeds 19: 1.
** Odds for significant difference between means exceeds 99: 1.

little or no influence on the concentration of these elements. The most efficacious washing treatment for the several elements involved is the combination of the detergent and acid. Iron was just as efficiently removed by the detergent alone. Copper and Zn were apparently partially removed by acid and partially by the detergent, since each of these removed substantial amounts of these elements and the lowest reproducible level was obtained with the acidulated-detergent washing. It is not possible to conclude, however, that the amounts of these elements remaining after washing are totally within the leaf. Although the proportion of Cu was greatly reduced by the acidulated-detergent wash, it seems unlikely that the remaining Cu was all internal. If so, it is from five to 10 times the concentration usually found in normal, unsprayed citrus leaves (**13**). Zinc, especially under sand culture conditions, frequently occurs in

TABLE IV

A COMPARISON OF THE APPARENT COMPOSITION OF SPRAYED* ORANGE LEAVES IN RESPECT TO CERTAIN MICRO-ELEMENTS AFTER VARIOUS WASHING AND GRINDING** TREATMENTS

Sample treatment	P.P.M. in dry leaves						
	Fe	Mn	Cu	B	Na	Zn	Al
Wiped with moist cheesecloth	57.7	27.3	209	169	513	208	133
Scrubbed in tap water	57.3	27.3	237	157	466	202	109
Scrubbed in 0.3N HCl	57.3	29.0	125	160	480	79	133
Scrubbed in Dreft	45.0	26.3	107	154	513	100	76
Scrubbed in Dreft (NG)	45.3	22.3	108	160	482	115	87
Scrubbed in Dreft + 0.3N HCl	45.0	22.7	67	165	517	62	65
Scrubbed in Dreft + 0.3N HCl (NG)	44.0	22.0	65	161	488	59	62
L.S.D. between any two values 0.05	4.9	2.4	46	N.S.	N.S.	44	15
L.S.D. between any two values 0.01	6.9	3.4	64	N.S.	N.S.	61	21

* Old sweet seedling trees sprayed with a nutritional spray containing copper and zinc a few weeks before sampling.

** All samples were ground in a semi-micro Wiley mill with a nickel-coated screen, except the two treatments marked (NG), which were pulverized in a porcelain mortar.

amounts from 50 to 100 p.p.m. It is possible, therefore, that the 60 p.p.m. found after the most drastic washing is largely absorbed Zn. Healthy leaves from commercial orchards, however, usually vary only from 20 to 40 p.p.m. (**12**). It appears doubtful that leaves previously sprayed with materials containing Fe, Cu, or Zn can be satisfactorily used for diagnostic purposes for these particular elements.

Manganese is inconsistent. Of several tests over the past few years, this is the only time that this element has been affected by washing treatment. It appears that some Mn contaminant was in the spray mixture, and this was affected by washing in much the same way as Fe.

Aluminum, under these conditions, did not behave in quite the same way as Fe. The spray appears to have influenced its removal in some way. The lowest values were obtained with the acidulated-detergent washing treatment.

The external contamination that we are most concerned with comes from colloidal particles, since sprayed leaves are usually avoided for trace-element analysis. Iron, Al, and Cu (perhaps Zn also) apparently are contributed by dust deposits on leaves. In the absence of spray residue, these elements appear to be removed as efficiently by the detergent alone.

Citrus leaves are glabrous and waxy and probably are capable of withstanding a more rigorous washing treatment than would more succulent leaves. It is possible that some mobile elements might be lost by leaching if the leaves become "waterlogged" with the detergent solution. With intact citrus leaves 8 to 10 minutes are required before internal wetting is evident. No evidence of leaching of even such a mobile ion as K has been found in the present studies, with six minutes as the maximum length of time that the leaves were in contact with the washing medium.

By carefully washing leaves of pear, corn, and tobacco in 0.3N HCl, JACOBSON (**8**) found a relationship between total iron and chlorophyll content. It is apparent from the present work that with citrus the action of a detergent is usually more efficient than acid when the leaves are thoroughly scrubbed on both surfaces.

MCGEORGE (**11**) recently found total Fe values ranging from 75 to 366 p.p.m. in Arizona citrus leaves of several varieties. Most values were greater than 150 p.p.m. No mention was made of a washing treatment for removal of external contamination. No difference existed between the green and chlorotic samples. The Mn values were all relatively low in comparison with those found with leaves from other citrus areas (**12**), and ranged from 10 to 32 p.p.m. The suggestion is made that the Mn deficiency pattern may be in reality an Fe toxicity condition.

In connection with a general survey of the composition of orange leaves from different localities, the present authors (**12**) collected 17 samples of newly matured leaves from different orchard locations in Arizona. These were rigidly selected for age, washed in Dreft solution, and handled in our regular procedure. The Mn values obtained were almost identical with those found by McGeorge. They ranged from 7 to 34 p.p.m. The total Fe values, however, were all quite low. One sample of leaves showing the iron chlorosis pattern showed 18 p.p.m. Fe. The over-all range of iron values for the 17 samples was only from 18 to 47 p.p.m. total Fe.

This wide discrepancy in Fe content along with others, such as the reported values for citrus in Florida (**5**) and California (**3**), leads to the suggestion that surface contamination has not received proper attention. Iron also increases with leaf age (**15**). In general, it seems possible that conclusions in regard to total Fe and iron-chlorosis and other Fe functions might become more harmonious if greater consideration were given to age of leaves sampled and removal of surface contamination.

From comparison with previous work (**3, 12**) it would appear that Fe and Mn are both present in inadequate amounts for proper leaf function

in the chlorotic leaves grown under highly acid soil conditions. Observations indicate that extensive root damage is associated with this type of tree condition. This would seem to indicate that the availability of either of these elements is not the primary cause of the disorder. The real cause is not yet understood but may be some toxic reaction which damages or kills most of the small feeder roots. Under these conditions Fe and Mn absorption, or translocation, is apparently depressed to the point that the leaves are inadequately supplied.

In regard to the general mineral composition of leaves low in total Fe and showing the iron-chlorosis pattern, the present results show increased K and lowered Ca in comparison with green leaves. Since this has been quite generally found with other plants, CHAPMAN (**4**) recently predicted that the same relationship would prevail in citrus. He further points out that all other known mineral-nutrient deficiencies that have been examined (except K) induce this same result. Higher N percentage in Fe-chlorotic leaves apparently is not widely recognized with the tree crops, although it was noted in relation to mottle-leaf (zinc deficiency) in California (**9**) and in leaves with lime-induced chlorosis in Arizona (**11**). The increased P and lowered Mg observed in chlorotic leaves in the present study are variations that were not found in the lime-induced chlorotic citrus leaves in Arizona (**11**).

There seems to be an over-all similarity between the composition of citrus leaves showing the "iron-chlorosis" pattern whether it occurs in acid soil, calcareous soil, or poorly drained soil. Iron and manganese both appear to be unusually low in such leaves. For example, a sample of Valencia orange leaves obtained in Texas and showing "iron-chlorosis" symptoms contained 11.6 p.p.m. of Mn and 40 p.p.m. of Fe (**12**). Likewise, a leaf sample obtained in Arizona representative of Valencia oranges associated with overirrigation (**11**) contained 18 p.p.m. of Fe and 8.5 p.p.m. of Mn (**12**). The deficiency pattern was the same. Therefore, it appears that with the disorders of tree crops called "iron chlorosis" or "lime-induced chlorosis," consideration should be given to manganese as well as to iron nutrition.

Summary

Leaves from orange trees on highly acid soil that were in a chlorotic and unthrifty condition were used as test material for studies on preparation of samples of leaves for analysis. Eleven chemical elements were determined. The various treatments tried for removing surface contamination consisted in wiping both surfaces with moist cheesecloth, scrubbing in tap water, in HCl, in a neutral detergent (Dreft), and in an acidulated detergent solution. The surface contaminants consisted chiefly of natural clay and organic dust particles, although a few tests were made with leaves sprayed with Fe, Cu, and Zn.

With unsprayed leaves total Fe, Al, and Cu were reduced to minimal values by scrubbing in a neutral detergent solution, followed by adequate rinsing. Sodium was absorbed from the detergent solution by chlorotic leaves but not by healthy ones. No other element tested was influenced by washing treatment.

With leaves that were sprayed with Cu and Zn in the presence of lime, acidulated detergent solution consistently gave the lowest analytical values for these elements. There is some doubt, however, that such treatment completely removes surface contaminations of this type.

Grinding the leaf samples in a mechanical mill did not appreciably influence the composition in regard to the elements determined.

Total iron of the leaves cleansed with the detergent showed a strong inverse relationship to the degree of iron chlorosis. Severely chlorotic leaves showed from about 10 to 15 p.p.m. total iron; mildly chlorotic leaves from about 18 to 30 p.p.m. iron; and non-chlorotic leaves from about 35 to 100 p.p.m. Newly matured leaves from healthy orange trees usually show from 50 to 70 p.p.m. The chlorotic leaves also had an abnormally low Mn content.

The general composition of these chlorotic orange leaves and the need for greater emphasis on sampling methods and preparation of samples for analysis are discussed.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES
BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
UNITED STATES DEPARTMENT OF AGRICULTURE
ORLANDO, FLORIDA AND BELTSVILLE, MARYLAND

LITERATURE CITED

1. BENNETT, J. P. Iron in leaves. Soil Sci. **60**: 91–105. 1945.
2. CAMP, A. F., and FUDGE, B. R. Some symptoms of citrus malnutrition in Florida. Florida Agric. Exp. Sta. Bul. 335. 1939.
3. CHAPMAN, H. D. Tentative leaf analysis standards. California Citrogr. **34**: 518. 1949.
4. CHAPMAN, H. D., and BROWN, S. M. Investigations of tissue analysis for diagnosing the nutrient status of orange trees with special reference to potassium. Hilgardia. (In press.) 1950.
5. FUDGE, B. R. Magnesium deficiency in relation to yield and chemical composition of seedy and commercially seedless varieties of grapefruit. Proc. Florida State Hort. Soc. **51**: 34–43. 1938.
6. GADDUM, L. W., CAMP, A. F., and REUTHER, W. Spectrographic studies of tissues and corresponding soils of normal and physiologically diseased horticultural crops. Florida Agric. Exp. Sta. Ann. Rpt. pp. 82–84. 1936.
7. GRIMMETT, R. E. R. Zn deficiency in citrus trees. New Zealand Dept. Agr. Ann. Rept. pp. 56–62. 1938.

8. JACOBSON, L. Iron in the leaves and chloroplasts of some plants in relation to their chlorophyll content. Plant Physiol. **20**: 233–245. 1945.
9. KELLEY, W. P., and CUMMINS, A. B. Composition of normal and mottled citrus leaves. Jour. Agr. Res. **20**: 161–191. 1920.
10. LINDNER, R. C., and HARLEY, C. P. Nutrient interrelations in lime-induced chlorosis. Plant Physiol. **19**: 420–439. 1944.
11. MCGEORGE, W. T. A study of lime-induced chlorosis in Arizona orchards. Arizona Agric. Exp. Sta. Tech. Bull. 117. 1949.
12. REUTHER, W., SMITH, P. F., and SPECHT, A. W. A comparison of the mineral composition of Valencia orange leaves from the major producing areas of the United States. Proc. Florida State Hort. Soc. **62**: (in press) 1949.
13. SMITH, P. F., REUTHER, W., and SPECHT, A. W. The influence of rootstock on the mineral composition of Valencia orange leaves. Plant Physiol. **24**: 455–461. 1949.
14. SMITH, P. F., and REUTHER, W. Seasonal changes in Valencia orange trees. I. Changes in leaf dry weight, ash, and macro-nutrient elements. Proc. Amer. Soc. Hort. Sci. **55**: (In press) 1950.
15. SMITH, P. F., REUTHER, W., and SPECHT, A. W. Seasonal changes in Valencia orange trees. II. Changes in micro-nutrient elements and carbohydrates in leaves. Proc. Amer. Soc. Hort. Sci. (Manuscript to be submitted).

UNIVERSITY OF FLORIDA LIBRARIES

GENERAL USES AND METHODS OF THERMISTORS IN TEMPERATURE INVESTIGATIONS, WITH SPECIAL REFERENCE TO A TECHNIQUE FOR HIGH SENSITIVITY CONTACT TEMPERATURE MEASUREMENT[1]

ROBERT B. PLATT AND JAMES N. WOLF

(WITH THREE FIGURES)

Received March 16, 1950

The thermistor (thermally sensitive resistor) provides a readily available instrument for temperature measurement and control inherently superior to instruments now in use. It is small, inexpensive, and yet highly sensitive to changes in temperature. Though widely used in industry and in the physical sciences, it has had but limited use in academic biological research.

This paper indicates some general properties, applications and methods of use of thermistors to investigations of temperature relationships, and describes a technique using a thermistor for high sensitivity contact thermometry.

Thermistors are sintered mixtures of specially prepared metallic oxides, which, when modified in suitable form with platinum alloy wire leads and arranged in a circuit, constitute semi-conductors whose electrical resistance varies rapidly with temperature. When the logarithm of the resistance is plotted against temperature, the calibration curve obtained may be considered as a straight line over small temperature intervals. Circuit designs for direct temperature indication may utilize ohmmeter or wheatstone bridge types. Forms technically suited to physiological thermometry include beads, rods, discs, and probes. Their temperature coefficients at 25° C vary from −3.4 to −4.4%/C°, their thermal time constants in still air from 1 to 70 seconds, their dissipation constants in still air from 0.1 to 10 mw/C°, and their maximum temperature from 100–300° C. (Thermistor Information Sheet: Physiological Thermometry, Issue 2, February 3, 1947; Western Electric Company.)

The chief advantage of a thermistor over either a thermocouple or a platinum resistance element is in its greater sensitivity, which permits the use of a more rugged and less expensive external indicating equipment. Two additional advantages of a thermistor over a thermocouple are: (1) its high impedance eliminates for practical purposes resistance problems in circuit design, thus permitting any length lead wires and (2) there is no need for a junction maintained at a reference temperature.

Thermistors have a wide variety of applications. While some of the

[1] A contribution from the Department of Botany of the University of Pennsylvania.

uses given here are not directly related to ecological investigations, the methods employed do provide positive suggestions for the development of techniques in this field. BECKER, GREEN, and PEARSON (1) have reviewed the basic properties and uses of thermistors. Some of the principal characteristics of presently available types of thermistors and information on their application to design problems by specific examples from industrial uses have been given by DOWELL (4). In this paper Dowell lists eleven basic thermistor applications. Under the basic heading of thermometry he includes temperature measurement, control and compensation, calorimetry, and temperature alarm devices, while under the heading of radiation thermometry he lists their use in bolometers, pyrometers, and infrared spectrometers. A remarkable example given for the latter group is the infrared bolometer developed during the war that utilized a flake form of thermistor so sensitive that it could detect the warmth of a human being a quarter mile away.

Thermistors have been used for body temperature measurements of animals and man. WILLIAMS and THOMPSON (7) used a disc thermistor in a moulded plastic holder for obtaining a continuous record of body temperature in man from the external auditory canal. DRUMMETER and FASTIE (5) developed a field instrument for the intravenous measurement of animal blood temperature in the vicinity of the heart, by using a 1 mm. diameter thermistor bead mounted on the end of a 5-French catheter. BENJAMIN and HORVATH (2) devised a means for mounting a 0.4 mm. diameter thermistor bead in catheters so small that they could be inserted through 19 gauge hypodermic needles for continuous temperature measurements in human joints, or used directly for intracardiac and intravascular observations.

COLMAN and HENDRIX (3) have used a thermistor as the sensing element in a fiberglas unit which, though primarily used for measurement of soil moisture, will indicate temperature. A technique for the application of thermistors to water thermometry and limnological investigations has been given by PLATT and SHOUP (6).

In the spring of 1947 an inexpensive instrument was needed to obtain high sensitivity contact temperature measurements in the field. A Western Electric Model D170575 thermistor, with lead wires attached on opposite sides of the bead, was decided upon. A subsequent model, V597, with lead wires attached on the same side, is easier to mount for this kind of measurement. The model used is a 0.4 mm. diameter glass coated spheroid with two 0.025 mm. diameter platinum alloy leads. It has a temperature coefficient at 25° C of $-3.4\%/C°$, a thermal time constant in still air of approximately 1 second, a dissipation constant in still air of 0.1 mw/C°, and a nominal resistance at 25° C of 2000 ohms.

The 0.025 mm. diameter leads were connected into the circuit by means of 0.5 mm. inside diameter nickel alloy tubing cut into convenient lengths.

(Nickel alloy tubing, temper #1, O.D. 0.0315", wall thickness 0.012", Superior Tube Company, Norristown, Pennsylvania.) Each thermistor lead was crimped into one end of a tube while the heavier circuit wire was soldered to the other end.

In designing a suitable mount for these tiny thermistors it is desirable that the material used have minimal thermal conductivity, high insulating quality, be easily applied or removed, be tough and durable, and have high adhesive properties. The material finally decided upon was a nitro cellulose cement known as Lamac-weld, used commercially for attaching resoles to shoes without the aid of nails or stitching. (Lamac-weld Waterproof Cement, Lamac Process Company, Erie, Pennsylvania.) This cement is soluble in acetone, can be applied smoothly in coats of not over 0.01 mm. in thickness, is transparent when dry, and has a very low thermal conductivity. When a heat differential in excess of 40° C was applied to a mounting of this material 1 cm. posterior to the sensing element, no change in the element resistance was observed within two minutes.

In preparing the mount the element and portion of the lead wires to be cemented were supported so as to be free from surface contact. The cement was applied with a dissecting needle in thin layers, allowing two to eight hours to dry between applications. The drying process may be speeded up by use of an oven heated 75° to 100° C. Thin applications are necessary to minimize distortion and possible damage resulting from shrinking of the cement on drying. This shrinking, however, may be used to good advantage, for by controlling the relative amounts of cement applied, the mounts may be "pulled" into various shapes. After three or four coats, the lead wires were webbed together and additional coats added until the desired form was attained. Acetone was used to perfect the final shape and to remove excess cement from the sensing element. For accuracy in unilateral contact measurements, only one half of the sensing element was exposed, while the other part, including the lead wires, was embedded for support. Various clips may be designed for this mounting to hold the element firmly against the substance measured. A thermistor mounting fastened in a spring wire clip designed for obtaining leaf temperatures is shown in figure 1.

The associated circuit (fig. 2) was designed to utilize a Leeds and Northrup single range portable potentiometer No. 7655 which was available in the laboratory. With this design, temperature measurements to 0.01° C are easily obtained. As indicated above, a wide variety of inexpensive circuits may be substituted.

The 0.4 mm. diameter thermistor bead provides an inexpensive means of obtaining accurate and rapid high sensitivity contact temperature indications within small spatial limits in the field. By the use of long lead wires such indications have been obtained in relatively inaccessible places

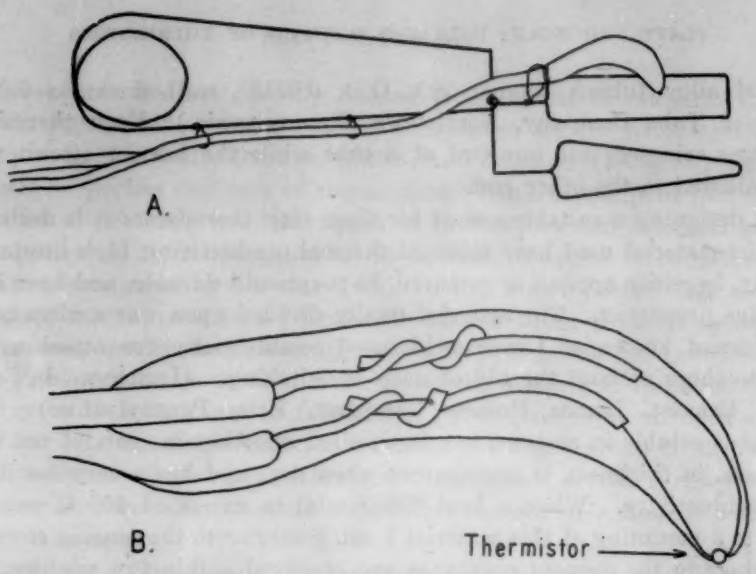

FIG. 1. A. Spring wire clip with thermistor and mounting for leaf temperature determinations. Overall length of clip 7.5 cm. B. Details of thermistor mounting. When properly pressed against the leaf surface the thermistor receives radiant energy only from the leaf itself while the mounting does not interfere with heat flow in the leaf. Effects on temperature caused by shielding of the leaf by the mounting are negligible.

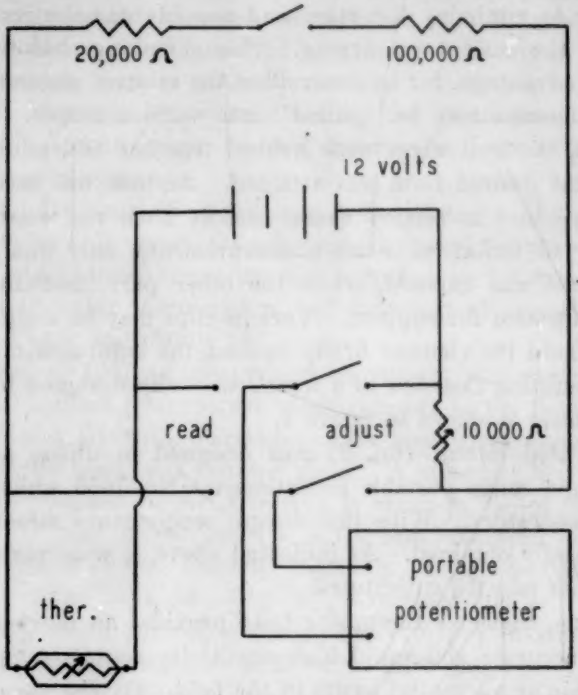

FIG. 2. Circuit design. Current through the thermistor is adjusted to .08 milliamperes (800MV drop across a 10,000 ohm variable resistor). The potential drop across the thermistor is then measured, either by adjustment of the potentiometer, or with precaution by galvanometer deflection.

UNIVERSITY OF FLORIDA LIBRARIES

as tree tops, cliff faces, and deep water. Some representative temperature determinations obtained by this method are presented in figure 3.

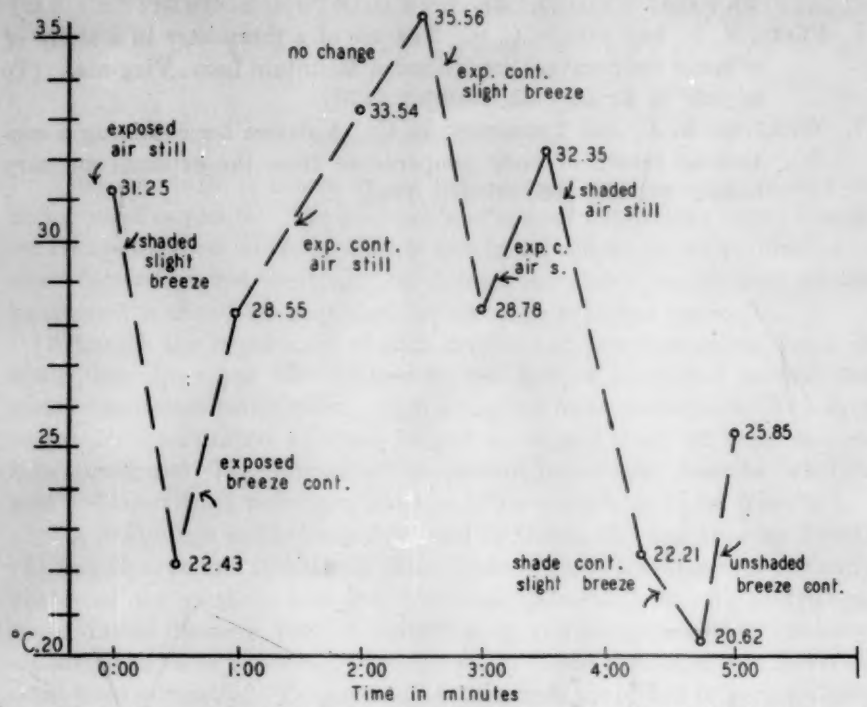

FIG. 3. Temperature changes in an oak leaf caused by changes in air movement and exposure. The leaf was oriented with upper side to sun. Circles denote temperature determinations. Arrows denote time of indicated changes in air movement and exposure.

DEPARTMENT OF BIOLOGY
EMORY UNIVERSITY
EMORY UNIVERSITY, GEORGIA
DEPARTMENT OF PHYSICS
UNIVERSITY OF KANSAS
LAWRENCE, KANSAS

LITERATURE CITED

1. BECKER, J. A., GREEN, C. B., and PEARSON, G. L. Properties and uses of thermistors—thermally sensitive resistors. Electrical Engineering **65**: Trans. 711–725. 1946.
2. BENJAMIN, J. M., JR., and HORVATH, S. M. Temperature measurement inside the body using a thermistor. Science **109**: 592–593. 1949.
3. COLMAN, E. A., and HENDRIX, T. M. The fiberglas electrical soil moisture instrument. Soil Science **67**: 425–438. 1949.
4. DOWELL, K. P. Thermistors as components open product design horizons. Electrical Manufacturing **42**: 84–91. 1948.

5. DRUMMETER, L. F., JR., and FASTIE, W. G. A simple resistance thermometer for blood-temperature measurements. Science **105**: 73–75. 1947.

6. PLATT, R. B., and SHOUP, C. S. The use of a thermistor in a study of summer temperature conditions of Mountain Lake, Virginia. (To appear in Ecology **31**. 1950.)

7. WILLIAMS, R. J., and THOMPSON, R. C. A device for obtaining a continuous record of body temperature from the external auditory canal. Science **108**: 90–91. 1948.

UNIVERSITY OF MICHIGAN LIBRARIES

THE CENTRIFUGE METHOD FOR MEASURING NECTAR YIELD[1]

C. A. Swanson and R. W. Shuel

(WITH TWO FIGURES)

Received October 29, 1949

Relatively little is known of the influence of environmental factors on nectar yield in plants. The problem is of special importance in the case of the forage legumes, where seed set is closely dependent on bee pollination if other factors are not limiting. In at least red clover, satisfactory nectar production is one of the requisites for ensuring sufficient bee visits.

Although the importance of such studies has been recognized, research along these lines has been limited by the lack of a suitable method for measuring nectar production. Such a method must combine rapidity with reasonable quantitative accuracy to permit critical study of these and related problems. The purpose of the present paper is to describe briefly a modified centrifuge technique which satisfies acceptably these criteria.

The centrifuge method was first used by Gubin (3) and later by Ewert (2) and Stapel and Götzache (5). None of these investigators critically evaluated the method, and few technical data, such as the centrifugal speed, radial distance, time of centrifuging, and completeness of recovery, are stated in these papers. A further study of the technique was therefore considered advisable. The method has also been simplified to permit direct volumetric determinations of yield, thereby eliminating the use of an analytical balance and considerably increasing the efficiency with which measurements can be made. All evaluations of the technique have been in terms of red clover but the method appears applicable to many other species.

Methods

Figure 1 illustrates the style of centrifuge tube used in this investigation. The microliters of nectar (centrifugate) are read directly from the height of the liquid in the calibrated capillary well of the centrifuge tube. For refractometer analyses, portions of the centrifugate may be easily withdrawn from the capillary with a micropipette, and the percentage of total solids (essentially glucose, fructose, and sucrose) readily determined. Thus rapid estimates of both volume and sugar content of nectar can be made. The centrifuge tubes are cleaned by forcing 70% alcohol through the capillaries with a micropipette and drying the flasks in an oven. Drying may be considerably expedited by first centrifuging the tubes in an inverted position.

[1] Papers from the Department of Botany, the Ohio State University, no. 518. Results of cooperative research between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agriculture Engineering, U. S. Department of Agriculture and the Department of Botany, Ohio State University, Columbus, Ohio.

A simple yet satisfactory support for holding the flower head in the centrifuge flask during centrifugation is illustrated in figure 1 (detail B). The support is made from stiff, light-gauge wire. During centrifuging, the flower head rests on the cross-wire, and is maintained in a vertical position by a pin inserted through the cork stopper into the base of the head. This method of support prevents the florets from becoming pressed too tightly against the glass or being deflected and held in a horizontal position during centrifuging. STAPEL and GÖTZACHE (**5**) and EWERT (**2**) simply suspended the flowers or inflorescences in the flasks by clamping the peduncles in a split stopper, but this method proved unsatisfactory in the present investigation. At a centrifugal force of 465 g. (standard in

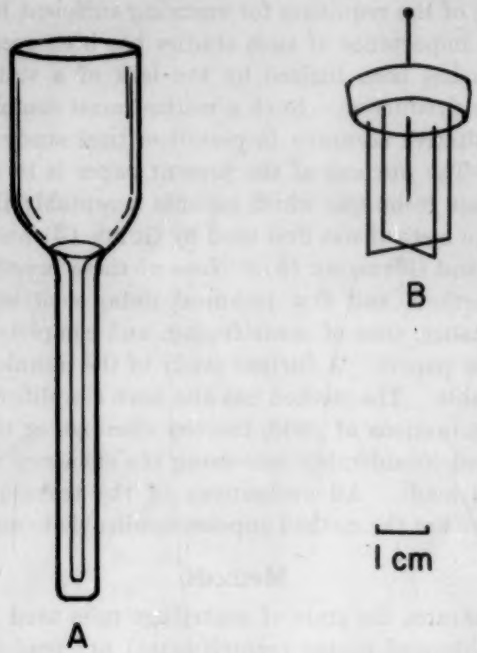

FIG. 1. A. Centrifuge tube. B. Stirrup support for holding clover head in centrifuge tube.

this study), the clover head stalks in many samples either broke or tore loose from the stoppers. The method of GUBIN (**3**) is not described.

Centrifuging was carried out in an International Clinical centrifuge at 2540 r.p.m. The flasks were placed in Cornell style metal tubes supported in a 4-place head. Since the radial distance approximated 7 cm., the flower heads were subjected to a centrifugal force of 465 g. Prior to centrifuging, the outer 4–6 mm. portion of the florets was trimmed off to facilitate extraction of the nectar (**5**).

The sensitivity of the method depends in part on the diameter of the capillary bore. For general work, capillaries of 1.5 mm. to 1.7 mm. bore

UNIVERSITY OF MICHIGAN LIBRARIES

size have been found satisfactory. Thus, for a nectar yield of 30 microliters, the column of centrifugate in a capillary of 1.50 mm. I.D. would be 16.9 mm. in length (1.77 μl./mm.). With a 10× binocular and ocular scale, readings may be easily made to 0.1 mm. The capillaries must be initially calibrated with water or mercury, and calibration tables prepared for each centrifuge flask. Readings should be taken to the middle portion of the meniscus to partially compensate for meniscus errors.

Sizeable errors may be introduced into the method by the inclusion of dew, precipitation, guttates from the sepals, or minute insects in the centrifugate. The insects can be blocked from the capillary by screens of suitable mesh. Even without this precaution, at least rough measurements are possible, for the insects are usually of lesser density than the nectar and separate out as an upper layer in the centrifugate. Dew and precipitation represent more serious errors and restrict the use of the method to periods of the day when neither is present in an interfering quantity.

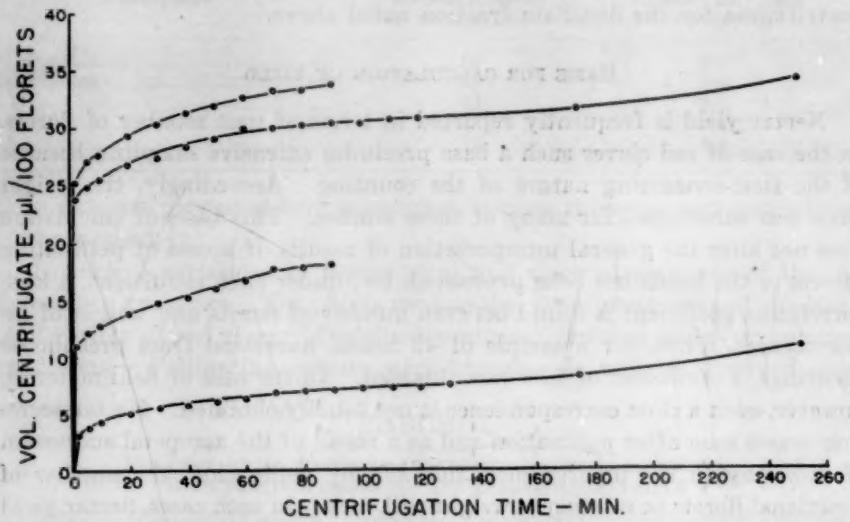

FIG. 2. Volume of centrifugate obtained as a function of centrifugation time. The nectar yield of the flowers is estimated to equal the volume of centrifugate obtained after six minutes of centrifuging. Explanation in text.

Experimental data

CENTRIFUGATION PERIOD

In order to measure the minimal centrifugation period necessary for essentially complete extraction of nectar, clover heads were centrifuged for periods up to four hours, readings being taken at intervals of two to 15 minutes. Representative curves, showing the amount of centrifugate obtained with time, are presented in figure 2. It will be observed that the volume of centrifugate increased continuously with increasing periods of centrifugation. Even four hours of centrifuging showed only an approximate approach to a limiting value.

It is very unlikely that such a time-yield relationship indicates continued extraction of nectar. The gradual increase in volume of centrifugate after four to six minutes of centrifuging is perhaps mainly due to evaporation of water from the clover head, and re-condensation on the surface of the centrifugate. Very similar curves were obtained when cotton wads, soaked in water, and compressed and tied to simulate clover heads, were centrifuged over a 50% sucrose solution. The continuing increase in yield with time of centrifugation is not due to the extrusion of liquid water from within the cell systems, for the centrifugal force component to which the cell water was subjected in these studies (465 g.) was trivial in comparison to the osmotic forces within the cells. On the basis of these studies, and others not here reported, a standard centrifugation period of six minutes was selected. Although the extraction of nectar is not 100% complete in this time, as is evident from the data in table II, further increments are negligible when correction is applied to the total centrifugate for the distillate fraction noted above.

Basis for calculation of yield

Nectar yield is frequently reported in terms of unit number of florets. In the case of red clover such a base precludes extensive sampling because of the time-consuming nature of the counting. Accordingly, the weight basis was substituted for many of these studies. This basis of calculation does not alter the general interpretation of results, if access of pollinating insects to the heads has been prevented, for, under such conditions, a high correlation coefficient is found between number of florets and weight of inflorescence. Thus, for a sample of 42 heads, harvested from greenhouse plantings, a coefficient of 0.96 was obtained. In the case of field material, however, such a close correspondence is not usually obtained. Nectar secretion ceases soon after pollination and as a result of the acropetal succession of flowering in the inflorescence, followed by pollination, the number of functional florets in relation to weight will vary. In such cases, nectar yield may be referred to various other bases, such as unit field area, unit number of heads, or unit number of plants.

Time interval between harvesting and centrifuging

The time required for preparing the clover heads for centrifugation varied from a half-hour to an hour, depending on the number of samples harvested. For field material, the transportation time to the laboratory must also be included. To determine the effect of moderate variations in time interval on the measured yield and sugar content, clover heads from the greenhouse were split lengthwise and one lot of half-heads was centrifuged one hour after harvesting, the other lot two hours after harvesting. The correlation coefficient for nectar yield between the respective lots of half-heads, based on a sample of 32 paired halves, was 0.73. This value is

UNIVERSITY OF MICHIGAN LIBRARIES

essentially the same as that obtained for the simultaneous centrifugation of paired halves (0.76 for a sample of 132 paired halves).

The results on sugar concentration were similar. No statistically significant differences in the per cent. of sugar in the nectar fractions from the two lots of flowers were found.

Scope of method

During the course of this work of evaluating the centrifuge technique, preliminary data have been secured on a number of problems. Some of these data are presented here mainly to illustrate the scope and possibilities of the method.

TABLE I

VARIATION IN NECTAR YIELD IN RED CLOVER WITH STAGE OF MATURITY OF INFLORESCENCE

MATURITY	NUMBER OF INFLORESCENCES IN SAMPLE	AVERAGE NECTAR YIELD (μL.) /100 FLORETS)
−1 day	15	8.5
Full bloom	55	9.4
+1	40	9.0
+2	18	6.4
+3	12	2.8

In table I variations in nectar yield and stage of maturity of the inflorescence are given. The plants used in this experiment were of the Kenland variety of red clover (*Trifolium pratense*) growing under greenhouse conditions. Pollinating insects were absent. It will be observed that under these conditions there was a decided decrease in nectar yield two or three days after reaching full bloom.

TABLE II

PER CENT. TOTAL SUGARS IN SUCCESSIVE FRACTIONS OF NECTAR. EACH FRACTION COLLECTED AND ANALYZED SEPARATELY

FRACTION	PER CENT. SUGAR IN SUCCESSIVE FRACTIONS OF NECTAR SAMPLE NO.				
	1	2	3	4	5
1st 2-min. fraction	61.1%	54.4%	70.2%	59.1%	68.0%
2nd 2-min. fraction	55.8	48.2	59.0	54.3	52.4
3rd 2-min. fraction	57.0	.....	48.8	55.2	.....
4th 2-min. fraction	54.6	43.2	.....	49.6	36.4
5th 2-min. fraction	42.2	.....	.....	.....	.....
6th 2-min. fraction	.....	.....	.....	44.2	.....

In table II data are presented showing the variation in per cent. sugars in successive fractions of nectar, collected and analyzed separately. It will be observed that in each case the per cent. sugar was highest in the first

two-minute fraction and with few exceptions declined significantly with each successive fraction. The effect of these more dilute fractions on the average per cent. sugars for the total sample is, of course, slight, due to the small volume of the fractions obtained after the initial few minutes of centrifuging. No general correspondence to the time-yield curve (fig. 2) was shown in this experiment.

Although marked variations occurred in the nectar yield of successive inflorescences taken on various days from the same plant, as would be expected in part from the varying environmental conditions, nonetheless, in terms of microliters nectar/100 florets or gram inflorescence weight, variants which were consistently "high yielders" and "low yielders" were distinguishable during the course of these studies. Thus for plant 14, the mean nectar yield was 2.4 ± 3.4 μl./100 florets; for plant 9, 5.9 ± 2.6 μl.; and for plant 24, 15.2 ± 10.2 μl. In general, however, plants with inflorescences yielding large nectar quantities produced fewer inflorescences per plant, with the result that a calculation of yield on a unit field area basis or per plant basis might result in an entirely different interpretation. Further studies of these reciprocal or correlative relationships are needed. Clones are being established of some of these variants (all of the Kenland variety) for further studies of yield in relation to genotype.

The average yield of nectar obtained in this investigation was of the order of 20 μl./100 florets as compared with an average yield of 32 μl./100 florets calculated from the data of STAPEL and GÖTZACHE (**5**). Since yields of 1 μl. can be determined in properly dimensioned capillaries, yields from as few as five to 10 florets are measurable under these conditions. The method, therefore, can be used for the investigation of relative yields from different florets in the inflorescence, and other similar types of problems.

Comparison of methods

In the course of this work, trial experiments with other methods of assay were made. For many purposes the micropipette method (**6, 7, 8**) is preferable, requiring only the simplest of equipment. With proper facilities, however, the volumetric centrifugation method is much faster, and for large scale sampling, quantitative recovery of nectar for yield data is more reliable. The micropipette method has the advantage, however, of not destroying the samples used for analysis.

Water extraction methods (**1, 4, 9**) on the other hand are subject to the serious limitation of providing data only on the sugar content of the nectar fraction. Although in general the quantity of sugars, calculated as glucose, recovered from the nectar was of the same order of magnitude as that calculated from centrifuge data, the precise value varied with the period of soaking. For example, in one experiment in which the inflorescences were submerged in 20 ml. water, and evacuated to remove entrapped air bubbles, the glucose equivalents of the acid hydrolyzed solutions amounted

UNIVERSITY OF MICHIGAN LIBRARIES

to an average of 12.4 mg./gram inflorescence after 20 minutes of soaking and 16.9 mg. after two hours, a gain of 36%. To what extent this gain represents continued recovery of nectar sugars or direct lixiviation of sugar from the cells of the plant tissue was not determined.

STAPEL and GÖTZACHE (5) obtained data on nectar yield in terms of both weight of centrifugate and height of the nectar column in the corolla tubes. It would be of interest to calculate a correlation coefficient between the two methods, but only averages for different treatments or seasons of planting are given in their paper. The data show that the calibration factor, *i.e.*, nectar yield in $\mu l.^2$/height of nectar column in mm., was highly variable, varying over 50% between certain treatments. To what extent this variability was due to environmental differences can only be surmised, for replicated data on similar treatments are not given. No high accuracy, however, can be expected from this method, for a variation of only 25 microns in the radius of the corolla tube would be equivalent to a percentage variation of approximately 20% in the calibration factor. Furthermore, it should be recognized that the method measures only the nectar which has accumulated in the corolla tubes. When the corolla is removed for measurement, an indeterminate fraction of the nectar remains in the calyx whorl. It is evident that this method is applicable only as a crude index to yield.

Summary

A volumetric centrifuge method of estimating nectar yield is described and evaluated. The volume of yield is determined directly from measurements of the height of the centrifugate in a calibrated capillary well attached to the centrifuge tube. Samples of nectar may be easily withdrawn from the capillary tube for refractometer analyses. The method, therefore, permits estimates of both volume of nectar and per cent. total sugars.

For red clover, a standard centrifugation period of six minutes at 465 g. was found satisfactory.

The authors are grateful to Mr. J. G. Dean, Jr., Division of Forage Crops and Diseases, BPISAE, U. S. Department of Agriculture for assistance in certain phases of the investigation.

DEPARTMENT OF BOTANY
OHIO STATE UNIVERSITY
COLUMBUS 10, OHIO

LITERATURE CITED

1. BONNIER, G. Influence du terrain sur la production du nectar des plantes. Ass. Fr. Av. Sci. **2**: 567–569. 1893.
2. EWERT, R. Der Blutennektar. Leipzinger Bienenzeitung. **54**: 173–176. 1939.

[2] Calculated from their data on milligrams yield divided by the specific gravity corresponding to the per cent. sugars in the centrifugate.

3. Gubin, A. F. Bestaubung und Erhöhrung der Samenernte bei Rotklee *Trifolium pratense* L. mit Hilfe der Bienen. Archiv. für Bienenkunde **17**: 209–264. 1936.
4. Kenoyer, L. A. Environmental influences on nectar secretion. Bot. Gaz. **63**: 249–365. 1917.
5. Stapel, Chr., and Götzache, O. Om nektarsekretion og kronrørslaengde m.m. hos rødkløvern under forckellige gødskningsforhold. Tidsskr. Planteavl. **46**: 267–297. 1941.
6. Vansell, G. H., Watkins, W. Q., and Bishop, R. K. Orange nectar and pollen in relation to bee activity. Jour. Econ. Ent. **35**: 321–323. 1942.
7. Vansell, G. H. Alfalfa nectar and the honey bee. Amer. Bee Jour. **83**: 106–107. 1943.
8. Vansell, G. H. Cotton nectar in relation to bee activity and honey production. Jour. Econ. Ent. **37**: 528–530. 1944.
9. Von Planta, A. Uber die Zusammensetzung einige Nectar-Arten. Zeitsch. Physiol. Chem. **10**: 227–247. 1886.

UNIVERSITY OF MICHIGAN LIBRARIES

BRIEF PAPERS

CYTOCHROME OXIDASE IN CORN EMBRYOS[1]

R. E. MAXWELL

Received January 16, 1950

In connection with a study of iron metabolism in corn, it was necessary to establish the presence of the cytochrome system in corn embryos.

Evidence has been presented for the functioning of the cytochromes in the embryos of wheat (**1**), oats (**2**), and barley (**3**). It would be logical to assume, therefore, that corn embryos also contain cytochrome oxidase. However, in view of the present state of knowledge of plant respiratory enzymes, it would be unwarranted to accept such an assumption without proof. A search of the literature failed to reveal any studies designed to indicate the nature of the terminal oxidase in corn embryos.

Air dry corn kernels of the hybrid WF9 × M14 were selected with regard to integrity and weight (250 ± 10 mg.). The seeds were rinsed briefly with 0.2% mercuric chloride and then flushed with a large volume of sterile water. Sterile technique was used throughout the experiments with whole kernels, and there has been no observable instance of mold or bacterial growth. Individual seeds were placed in Warburg flasks lined on the bottom with filter paper. For the control vessels, 0.5 ml. sterile water was added. The same amount of various solutions containing respiratory inhibitors was added to the experimental flasks. The concentrations of the inhibitors were selected as being likely to show appreciable inhibition of iron or copper containing enzymes, the selections being based on concentrations used in studies referred to in the bibliography. All solutions were adjusted to pH 7.0. In the center wells, 0.2 ml. 20% KOH was used with a fluted section of filter paper, except for the cyanide inhibition study, in which KCN was used as the carbon dioxide absorbent (**4**). Oxygen uptake was measured, in an atmosphere of air and at 37° C, for 60 minute periods at various intervals, usually extending to 25 hours. By this time the control seeds exhibited a large oxygen consumption and roots 10 to 11 mm. in length.

In table I it may be seen that the profound effects of cyanide and azide leave little doubt as to the involvement of a heavy metal enzyme. In these cases germination was completely arrested. The results obtained with phenylthiourea and diethyldithiocarbamate suggest that the copper enzymes tyrosinase and ascorbic acid oxidase might not be involved in the corn seed's respiration at this stage of development (**5, 6**). However, ger-

[1] Journal paper No. J-1705 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1049.

mination did not proceed at the normal rate in the presence of the latter two inhibitors, since the root length was reduced to 3 to 6 mm. at the end of 25 hours.

These inhibition effects naturally pertain to the respiration of the endosperm as well as of the embryo, but one would expect that major portion of the respiratory activity to be centered in the growing and differentiating embryo. In the case of wheat, it has been stated that the magnitude of embryo respiration is 10 to 15 times greater than that of the endosperm (**7**).

In order to obtain more direct evidence for the activity of the cytochrome system in the embryos, homogenates were prepared from embryos with 10 to 11 mm. roots. Ten embryos (free of endosperm) were selected which had a total weight of 1.0 ± 0.1 gm. These were crushed in a mortar with 2.0 ml. of ice cold sodium phosphate buffer, 0.1 M, pH 7.0, another

TABLE I

OXYGEN UPTAKE BY CORN KERNELS

SOLUTION	MM.* O_2 PER SEED DURING INDICATED HOUR*		
	6–7	18–19	24–25
Controls	31 (25–33)	146 (127–162)	166 (140–184)
0.002 M PTU†	35 (21–54)	157 (142–158)	165 (159–175)
0.01 M DDC††	39 (35–46)	104 (97–108)	125 (107–141)
0.005 M NaN_3	3 (1–4)	6 (4–7)	4 (1–8)
0.001 M KCN	0	0	0

* Average is given first, followed by range of six determinations.
† Phenylthiourea.
†† Sodium diethyldithiocarbamate.

2.0 ml. of the buffer was added and the suspension was homogenized in a glass apparatus (**8**). Aliquots of these preparations were used for the experiments summarized in table II. Oxygen uptake from an atmosphere of air was measured in a conventional Warburg apparatus at 37° C and a shaking rate of 120 two centimeter strokes per minute. The cytochrome c used was prepared from horse heart by the method of KEILIN and HARTREE (**9**). The iron content of the preparation, as determined by the method of DRABKIN (**10**), was 0.28%. The concentrations of solutions of the pigment were determined in a Beckman quartz spectrophotometer at 550 mμ after reduction with hydrosulphite, using the extinction coefficient reported by THEORELL and AKESSON (**11**). Higher concentrations than that shown in the table did not increase the oxygen uptake, so that it may be assumed that the oxidase present in the homogenate was saturated at this level. In all of the experiments reported, the rate of oxygen uptake was essentially linear with time over the 60 minute period.

Initially, ascorbic acid was investigated as a reducing agent for the added cytochrome c (**12**). However, as indicated in the table, there was

UNIVERSITY OF MICHIGAN LIBRARIES

TABLE II

OXYGEN UPTAKE BY CORN EMBRYO HOMOGENATES*

FLASK CONCENTRATION OF ADDITIONS	MM.³ O_2 UPTAKE PER HOUR†
(1) None	4 (2–9)
(2) 0.00003 M cytochrome c	10 (9–12)
(3) 0.01 M p-phenylenediamine	51 (48–58)
(4) As in (3), but homogenate boiled	26 (20–31)
(5) 0.00003 M cytochrome c + 0.01 M p-phenylenediamine	148 (132–174)
(6) As in (5), but homogenate boiled	21 (20–22)
(7) As in (5), + 0.001 M KCN	22 (18–25)
(8) As in (5), + 0.005 M NaN_3	26 (22–31)
(9) 0.02 M ascorbate + 0.00003 M cytochrome c	357 (310–406)
(10) As in (9), but homogenate boiled	249 (200–299)
(11) 0.0025 M 3,4-dihydroxyphenyl-L-alanine	5 (0–8)
(12) 0.003 M p-cresol	0

* Each flask contained 1.0 ml. 0.1 M sodium phosphate buffer, pH 7.0; 0.5 ml. homogenate; 0.2 ml. 20% KOH and filter paper in center cup; and water to make a total volume of 3.0 ml. Substrates tipped from side arm after temperature equilibration.

† Each value is the average of at least three determinations, the range being indicated in parentheses.

a high rate of non-enzymatic catalysis of the oxidation of ascorbic acid, and in further experiments p-phenylenediamine was used. It should be emphasized that the conditions used in these experiments are not necessarily optimum. The results show, nevertheless, that addition of the specific substrate for cytochrome oxidase, reduced cytochrome c, causes a marked stimulation of oxygen uptake by the homogenate. Furthermore, this increase in activity is substantially abolished by cyanide and azide, two known inhibitors of cytochrome oxidase.

Under the conditions of these experiments, no significant cresolase or polyphenol oxidase activity was apparent.

CHEMISTRY SECTION
IOWA AGRICULTURAL EXPERIMENT STATION
AMES, IOWA

LITERATURE CITED

1. GODDARD, D. R. Cytochrome c and cytochrome oxidase from wheat germ. Amer. Jour. Bot. **31**: 270–276. 1944.
2. ALBAUM, H. G., and EICHEL, B. The relationship between growth and metabolism in the oat seedling. Amer. Jour. Bot. **30**: 18–22. 1943.
3. MERRY, J. and GODDARD, D. R. A respiratory study of barley grain and seedlings. Proc. Rochester Acad. Sci. **8**: 24–44. 1941.
4. LATIES, G. G. Limitations of the use of potassium hydroxide-potassium cyanide mixtures in manometric studies. Jour. Biol. Chem. **177**: 969–974. 1949.
5. DUBOIS, K. P., and ERWAY, W. F. Studies on the mechanism of action of thiourea and related compounds. Jour. Biol. Chem. **165**: 711–721. 1946.

6. Stotz, E., Harrer, C. J., and King, C. G. A study of "ascorbic acid oxidase" in relation to copper. Jour. Biol. Chem. **119**: 511–522. 1937.
7. Muller, D. and Holm, F. Die Atmung des Gräserendosperms. Planta **32**: 596–599. 1941.
8. Potter, V. R., and Elvehjem, C. A. A modified method for the study of tissue oxidations. Jour. Biol. Chem. **114**: 495–504. 1936.
9. Keilin, D. and Hartree, E. F. Purification and properties of cytochrome c. Biochem. Jour. **39**: 289–292. 1945.
10. Drabkin, D. L. Spectrophotometric studies. VIII. The microdetermination of iron in cytochrome c and hemin preparations. Jour. Biol. Chem. **140**: 387–396. 1941.
11. Theorell, H., and Akesson, A. Studies on cytochrome c. I. Electrophoretic purification of cytochrome c and its amino acid comp. Jour. Amer. Chem. Soc. **63**: 1804–1811. 1941.
12. Schneider, W. C., and Potter, V. R. The assay of animal tissues for respiratory enzymes. II. Succinic dehydrogenase and cytochrome oxidase. Jour. Biol. Chem. **149**: 217–227. 1943.

UNIVERSITY OF MICHIGAN LIBRARIES

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE INVERTASE, PHOSPHORYLASE AND PECTIN METHOXYLASE ACTIVITY IN THE STEMS AND LEAVES OF THE RED KIDNEY BEAN PLANTS[1]

W. B. Neely, C. D. Ball, C. L. Hamner, and H. M. Sell

Received May 11, 1950

Introduction

The formation of proliferated stem tissue in the red kidney bean plant induced by 2,4-dichlorophenoxyacetic acid has aroused considerable interest. Sell, Luecke, Hamner and Taylor (**5, 8**) have reported large differences in protein, carbohydrates, lipides, amino acids, vitamins and ash content in the normal and the tumorous stem tissue of the red kidney bean plant produced by 2,4-D.[2] Further studies by Weller, Luecke, Hamner and Sell (**9**) have shown that these abnormal differences in chemical composition occurred primarily in the proliferated stem tissues. Recent observations by Felber (**1**), and Neely, Ball, Hamner and Sell (7) have disclosed enhanced activity for peroxidase and alpha and beta amylase, respectively, in the tumorous stem tissues. The purpose of the present communication is to report the activity of other enzymes in analogous material.

Method and results

Seeds of a certified strain of the red kidney bean plants were selected for uniformity of size and planted in 4-inch pots in the greenhouse in July 1949. Each pot contained two plants that were treated when the first trifoliate leaves were expanding, approximately 10 days after planting. Four replications of 100 plants each were used from which to obtain material of treated and non-treated plants (controls). Application of 2,4-D was made by applying one drop (.05 ml.) of a 1000 p.p.m. solution to the base of the blade of one of the primary leaves. The plants were harvested six days after treatment at which time the stem tissue had proliferated considerably but yet showed no signs of necrosis. The material was air dried at 30° C in the dark and then segregated into leaf, root and stem tissue. The hypocotyl, first internode and leaf petioles were grouped together as stem tissue. After drying, the material was ground to pass through a 60 mesh sieve, and then stored in a glass jar at room temperature and used as needed.

[1] Journal Article No. 1155 Michigan Agricultural Experiment Station, East Lansing.

[2] A 0.1% solution of the sodium salt of 2,4-dichlorophenoxyacetic acid acidified with orthophosphoric acid to pH 3 and abbreviated in this paper as "2,4-D."

Invertase was determined by using a modification of WILLSTATTER's (10) method. A variety of solvents such as salt solutions and water at various temperatures and extraction times were employed for extraction. However, none of the leaf or proliferated stem tissue showed any invertase activity. This would indicate that the hydrolysis of sucrose to hexose sugars by invertase is absent in the tissues of the young plants.

The procedure of KERTESZ (4) was employed for the determination of pectin methoxylase. The results in table I show that the activity of pectin methoxylase is approximately twice as great in the normal stem tissue as in the tumorous tissue of the red kidney bean plant. The same trend was also noted in the leaves of the treated and non-treated plants. The activity of the other pectic enzymes was not determined but if the ultimate breakdown of protopectin to methanol and galacturonic acid is a series of equilibrium reactions then the increase in pectin methoxylase

TABLE I

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ON THE PECTIN METHOXYLASE* ACTIVITY IN THE STEMS AND LEAVES OF THE RED KIDNEY BEAN PLANTS

ENZYME	SAMPLE	STEMS		LEAVES	
		TREATED	NON-TREATED	TREATED	NON-TREATED
Pectin Methoxylase	1	6.20	15.81	3.10	7.90
	2	7.44	16.10	2.79	8.05
	3	8.96	16.27	3.10	8.20
	4	7.90	16.11	2.94	8.05

* Pectin methoxylase unit is defined as the number of milligrams of methoxyl split off by one gram of material in 30 minutes at 30° C.

activity would likely be associated with an increase in the breakdown of the protopectin in the cell wall. This change has been observed by MURRAY and WHITING (6) in the histological studies of the tissue of young plants treated with 2,4-D.

Phosphorylase activity was measured by a method given by GREEN and STUMPF (3). The data in table II show that the phosphorylase activity in the tumorous stem and leaf tissue of the treated plants is much less than in the normal tissue of the control plants. Although a phosphatase acting on D-glucose-l-phosphate has not been reported to occur in the tissue of the bean plants, the possibility existed that the liberation of the phosphate might be due to such a phosphatase. Therefore, a determination was made for reducing sugar by the FOLIN and WU method (2) before and after enzymatic hydrolysis to ascertain whether there was an increase in reducing sugars. The amount of reducing sugar remained constant and showed that the liberation of phosphate was due to a phosphorylase enzyme converting the D-glucose-l-phosphate to a corresponding glucose polymer. The decrease in phosphorylase activity confirms the work of previous workers (8) that treatment of plants with 2,4-D dis-

UNIVERSITY OF MICHIGAN LIBRARIES

torts the carbohydrate metabolism in the cells. Since both the degradation and synthesis of starch have been observed to be reactions catalyzed by a phosphorylase and since the products of glycolysis of starch may enter the carboxylic acid cycle and would, therefore, be closely related to the synthesis of such important amino acids as aspartic and glutamic acids, it is believed that the effect of 2,4-D on phosphorylase activity may be an important point in the explanation of the basis of the distorted metabolism observed.

Summary

1. Invertase activity was absent in both the proliferated stem and leaf tissue of the red kidney bean plants treated with 2,4-dichlorophenoxyacetic acid and the controls.

TABLE II

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ON THE PHOSPHORYLASE* ACTIVITY OF THE STEMS AND LEAVES OF THE RED KIDNEY BEAN PLANTS

ENZYME	SAMPLE	STEMS		LEAVES	
		TREATED	NON-TREATED	TREATED	NON-TREATED
Phosphorylase	1	5.35	9.32	0.43	5.00
	2	5.91	8.76	.73	3.98
	3	5.68	8.98	.77	2.78
	4			.51	3.16

* Units are expressed as phosphorylase units where one unit has been defined as the amount of enzyme which catalyzes the liberation of 0.1 mg. of inorganic phosphorus from D-glucose-l-phosphate in three minutes at 38° at pH 6.

2. Pectin methoxylase activity increased in both the proliferated stem and leaf tissue of the treated plants.

3. Phosphorylase activity decreased in both the proliferated stem and leaf tissue of the 2,4-D treated plants.

4. There was no indication of the presence of a phosphatase acting on D-glucose-l-phosphate.

DEPARTMENTS OF AGRICULTURAL CHEMISTRY, CHEMISTRY, AND HORTICULTURE
MICHIGAN STATE COLLEGE
EAST LANSING, MICHIGAN

LITERATURE CITED

1. FELBER, I. M. The formation of protuberances of bean leaves in response to 2,4-D treatments. Amer. Jour. Bot. **35**: 555–558. 1948.
2. FOLIN, O. The determination of sugar in blood and in normal urine. Jour. Biol. Chem. **67**: 357–370. 1926.
3. GREEN, D. E. and STUMPF, P. K. Starch phosphorylase of potato. Jour. Biol. Chem. **142**: 355. 1942.
4. KERTESZ, Z. I. Determination of pectin-methoxylase activity. Jour. Biol. Chem. **121**: 589–597. 1937.

5. Luecke, R. W., Hamner, C. L., and Sell, H. M. Effect of 2,4-dichlorophenoxyacetic acid on the content of thiamine, riboflavin, nicotinic acid, pantothenic acid and carotene in stems and leaves of red kidney bean plants. Plant Physiol. **24**: 546–548. 1949.
6. Murray, M. A., and Whiting, A. G. A comparison of the effectiveness of 2,4-D and four of its salts in inducing histological responses in bean plants. Bot. Gaz. **109**: 13–39. 1947.
7. Neely, W. B., Ball, C. D., Hamner, C. L., and Sell, H. M. Effect of 2,4-dichlorophenoxyacetic acid on the alpha and beta amylase activity in stems and leaves of red kidney bean plants. Science **111**: 118. 1950.
8. Sell, H. M., Luecke, R. W., Taylor, B. M., and Hamner, C. L. Changes in chemical composition of the stems of red kidney bean plants treated with 2,4-dichlorophenoxyacetic acid. Plant Physiol. **24**: 295–299. 1949.
9. Weller, L. E., Luecke, R. W., Hamner, C. L., and Sell, H. M. Changes in chemical composition of the leaves and roots of red kidney bean plants treated with 2,4-dichlorophenoxyacetic acid. Plant Physiol. **25**: 289–293. 1950.
10. Wilstatter, R., and Kuhn, R. Über Masseinheiten der Enzyme. Ber. **56**: 509. 1923.

UNIVERSITY OF MICHIGAN LIBRARIES

NOTES

THE STATUS OF PLANT PHYSIOLOGY IN THE UNITED STATES: THE NUMBER, INTERESTS AND RATE OF TRAINING OF PLANT PHYSIOLOGISTS

COMMITTEE FOR PROFESSIONAL STATUS AND TRAINING[1]

Introduction

Considerations of the status of plant physiology in the United States have been hampered by the fact that information is not available concerning the number of plant physiologists active in the field at the present time. Information on the current rate of preparation of qualified plant physiologists on the professional level has also been lacking. This report concerns an attempt made by the committee on Professional Status and Training of the American Society of Plant Physiologists to gain some insight into both of these questions. Incident to the obtaining of this data it proved possible to obtain other information also. It has long been recognized in a qualitative way that the great bulk of the work in plant physiology is carried on in the name of horticulture, agronomy, and other phases of plant science. A quantitative estimate of the number of plant physiologists working in plant physiology but carried out under these varied formal designations has now been obtained. The quantitative distribution of workers in the varied aspects of plant physiology has also been investigated.

Method

During the month of April, 1949, a questionnaire was prepared and sent to 140 individual members of the American Society of Plant Physiologists located at universities and colleges, agricultural experiment stations, federal laboratories and certain industrial laboratories located in the United States. Of this number 136 questionnaires were returned. The individuals selected for participation in the survey were informed that only one form would be sent to each institution and each was requested to include in his tally all persons at that institution working specifically in the field of plant physiology as well as those in other departments but working in fields of which plant physiology is an important part. In this way it was hoped to avoid replicate enumeration of workers in a particular institution. The method did however exclude individuals located at in-

[1] H. B. Tukey, Michigan State College; Cecil Wadleigh, U. S. Regional Salinity Laboratory, Riverside; T. C. Broyer, University of California, Berkeley; K. V. Thimann, Harvard University, Cambridge; James Bonner, California Institute of Technology, Chairman.

stitutions other than those surveyed. Of the 753 members of the Society at the time of the survey, 432 or 57% were accounted for by the survey. Since care was taken to include major centers of plant science work, academic, governmental, and industrial, the members not accounted for are principally those working individually or in very small groups. The questions included in the questionnaire concerned the numbers of people carrying on research, teaching, or other activities, both specifically in plant physiology and in closely allied fields in which plant physiology is an important part. Separate tallies were made for the professional, sub-professional and graduate student levels.

The exact definition of what constitutes work not officially called plant physiology but of which plant physiology is an important part was in a large measure left to the individual census-taker. It is clear from the response however that work in plant physiology is carried on very commonly in groups otherwise devoted to pathology, agronomy, horticulture,

TABLE I

NUMBER OF PLANT PHYSIOLOGISTS RECORDED IN THE SURVEY DIVIDED ACCORDING TO PROFESSIONAL STATUS

	WORKING SPECIFICALLY IN PLANT PHYSIOLOGY	WORKING IN FIELDS CLOSELY ALLIED TO PLANT PHYSIOLOGY
Professional level	286	1170
Sub-professional level	138	353
Graduate students	262	1004
Total	686	2527
Members of A.S.P.P.	219	213
Members of A.S.P.P. (per cent of total)	31.9	8.4

pomology, truck crops, and even forestry. Plant physiology carried on in the service of these various fields will hereafter be referred to for convenience as applied plant physiology. It should be emphasized that a clear distinction is here drawn between any particular agricultural field and the study of plant physiology in connection with this field. Thus a plant physiologist or agronomist working on a plant physiological problem in an agronomy department would be included in this survey as an applied plant physiologist. An agronomist working on a soils problem in an agronomy department would not be included.

Results

Table I presents a summary of the data on numbers of workers in plant physiology in the United States broken down according to professional status and according to official designation. As mentioned above, the survey is incomplete since of the 753 members of the American Society of

Plant Physiologists at the time of the survey only 432 or 57% were accounted for. Since questionnaires were sent to every major university and agricultural college as well as to major governmental and industrial research centers, it is believed that the survey presents a reasonably correct picture of the larger centers of activity. The data of table I reveal that there are roughly four times as many workers in applied plant physiology as in plant physiology proper. This difference applies not only to professional workers but also to graduate students. It is clear therefore that the great preponderance of work in plant physiology today is actually carried on as applied plant physiology and under titles other than plant physiology.

A second important fact revealed by the data of table I is that there are many more plant physiologists than there are members of the American Society of Plant Physiologists. Only 32% of the plant physiologists were found to be members of the Society. Although figures were not obtained, it seems safe to assume that most of the 219 members of the society recorded as "official" plant physiologists would be found among the 286 professional workers, a relatively high proportion. In any case, much remains to be done in encouraging membership in the Society, probably

TABLE II

GRADUATE STUDENTS EXPECTED TO OBTAIN THE PH.D. DEGREE:

	SPECIFICALLY IN PLANT PHYSIOLOGY	IN CLOSELY ALLIED FIELDS
By July 1, 1950	119	153
By July 1, 1951	108	133

particularly in the lower levels of professional status. Of the 2527 applied plant physiologists tabulated in the survey only 213 or 8.4% were found to be members of the A.S.P.P. Despite the low proportion of membership, this group contributes almost as many total members to the society as do the plant physiologists proper. Here again it is clear that a great many workers in applied plant physiology are not members of the society which would presumably most uniquely fit their scientific interests.

Table I shows that there is a great number of graduate students both in plant physiology and in applied plant physiology. For every professional worker in the field there appears to be approximately one graduate student in training. Table II indicates that slightly over 100 Ph.D. degrees are expected both in 1950 and in 1951 in plant physiology and a slightly higher number in applied plant physiology. It is interesting to note that the proportion of presumptive Ph.D.'s is approximately three times as high among plant physiologists as among applied plant physiologists.

The questionnaire included one question as to the fields of major in-

terest represented. Although this question was not answered by all respondents, still the number of replies was sufficiently large to yield an interesting picture of the distribution of effort in plant physiology at the present time. More than 50 different fields of interest were indicated for the 1622 workers covered. These 50 fields were therefore combined into 11 broader categories. Thus the category plant biochemistry includes work reported as intermediary metabolism, protein metabolism, metabolism, respiratory systems, phosphorolytic enzymes, enzymes, and plant constituents. The data given in table III show that plant nutrition continues to

TABLE III

DISTRIBUTION OF EFFORTS OF WORKERS IN PLANT PHYSIOLOGY AND ALLIED FIELDS

	SPECIFICALLY IN PLANT PHYSIOLOGY		IN ALLIED FIELDS	
	No.	%	No.	%
Physiology of microörganisms	31	6.8	229	19.5
Developmental physiology	14	3.1	19	1.6
Translocation	6	1.3	0	0.0
Plant growth substances	93	20.7	22	1.9
Nutrition	107	23.8	163	13.9
Photosynthesis	38	8.4	0	0.0
Biochemistry	52	11.6	124	10.6
General physiology of a specific crop	27	6.0	426	36.3
Radioactivity and radiation effects	9	2.0	0	0.0
Water relations	13	2.9	22	1.9
General and teaching	60	13.3	167	14.2
Total	450	99.9	1172	99.9

command the interest of more plant physiologists than any other single subject, comprising about 24% of all plant physiologists reported. The study of plant growth substances, a field hardly 20 years old, is however a close second with 21% of all plant physiologists reported. All other fields are represented by substantially smaller proportions of the total, biochemistry and photosynthesis commanding but 12% and 8% of the total, respectively.

The distribution of interests among the applied plant psysiologists appears to be substantially different from the distribution among the plant physiologists proper. More than one third of the applied plant physiologists have interests directed toward the physiology of a specific plant or crop rather than toward a specific subject or field of physiology. The second largest group with 19.5% of the total is constituted by workers with interests in the physiology of microörganisms, a group largely represented by physiologists working in plant pathology. Plant nutrition with 14% of the total is also a substantial interest of the applied plant physiologists. Interestingly enough, plant growth substances appear to be only yet of minor interest to applied plant physiologists, despite the very considerable amount of work being carried on with 2,4-D.

UNIVERSITY OF MICHIGAN LIBRARIES

According to the definition given above, applied plant physiology is considered as plant physiology carried on in the service of some other field or department of plant science. Table IV gives data on the distribution of applied plant physiologists (professional grade men) according to formal designation of field or department. It may be seen that applied plant physiologists are relatively uniformly distributed in horticulture, pathology, agronomy and genetics, and soils.

TABLE IV

DISTRIBUTION OF WORKERS IN APPLIED PLANT PHYSIOLOGY ACCORDING TO FORMAL DESIGNATION AS TO FIELD OR DEPARTMENT

FIELD OR DEPARTMENT	No.	%
Horticulture	225	26.7
Pathology	229	27.2
Agronomy and genetics	225	26.7
Soils	163	19.4
Total	842	100.0

Finally, it is of interest to know the way in which work in plant physiology is distributed between universities and colleges, the U. S. Department of Agriculture, and industry. For the purposes of this tabulation, universities, colleges and experimental stations have been pooled since it proved difficult to disentangle relationships in institutions which have all three represented on one campus. The U. S. Department of Agriculture is

TABLE V

DISTRIBUTION OF PLANT PHYSIOLOGISTS ACCORDING TO EMPLOYER. (ONLY PROFESSIONAL WORKERS ARE CONSIDERED IN THIS TABLE.)

EMPLOYER	PLANT PHYSIOLOGISTS		APPLIED PHYSIOLOGISTS	
	No.	%	No.	%
University or Experiment Station	180	63.0	924	81.5
U. S. Department of Agriculture	45	15.7	127	11.2
Other Federal Government Agencies	15	5.2	28	2.5
Chemical Industry	6	2.1	16	1.4
Agricultural Industry	5	1.8	12	1.1
Miscellaneous	35	12.2	26	2.3
Total	286	100.0	1133	100.0

treated as one category; other government agencies, such as AEC, Department of National Defense, etc. are pooled as a separate category. Chemical industry does not include the fertilizer industry which is lumped with private agricultural research departments under the heading agricultural industry. The category miscellaneous includes plant physiologists working in medical research institutes and private non-profit research institutes.

The data of table V show that of the plant physiologists covered by this

survey 63% work in academic institutions and the associated experiment stations. An even larger proportion of the applied plant physiologists, 81.5%, fall in the same category. The U. S. Department of Agriculture constitutes the second category in both instances although the numbers employed are far smaller than those in academic institutions. The number of plant physiologists in industry, either chemical or agricultural, is very small, consisting of less than 4% for both plant physiologists and applied plant physiologists.

Discussion

The following general points may be made:

1. Less than one third of the plant physiologists of the United States are members of the A.S.P.P. Only about 8.5% of the applied plant physiologists are members of the society. If most of the membership is concentrated in the upper professional level as seems probable the figures at this level could rise to a maximum of 77% and 18% respectively.

2. A total of 286 professional grade plant physiologists and 1170 applied plant physiologists were accounted for in the survey. Extrapolation on the basis of the fraction of the membership of the A.S.P.P. accounted for suggests that there may be a total of the order of 500 professional grade plant physiologists and 2000 professional grade applied plant physiologists active in the United States at the present time.

3. Over 100 Ph.D. degrees are expected to be granted in plant physiology in each of the years 1950 and 1951. In applied plant physiology 153 and 133 Ph.D. degrees will be granted in these two years.

4. Plant physiologists and applied plant physiologists alike are employed principally in universities, colleges and experiment stations. The U. S. Department of Agriculture represents quantitatively the next most important employer, with private industry employing only an extremely small proportion of the total.

The data presented in this report have been collected as a preliminary to more detailed considerations concerning the professional training and status of plant physiologists. The data do of themselves however raise several important questions. Perhaps the most pressing of these relates to the number of graduate students now in training. This number is large in proportion to the present total body of plant physiologists. A great many will presumably go, as in the past, into various phases of applied plant physiology. If there are about 2500 plant physiologists (pure and applied), each with an average useful life of 35 years, it follows that the rate at which students should be trained for replacement purposes is about 75 per year. Any number in excess of 75 can be absorbed only by expansion of the field. Of course there has been a temporary shortage of physiologists due to suspension of training during the war. Undoubtedly also there is a certain amount of expansion. Even so, how rapidly can the existing agencies expand to absorb these new professional grade plant

UNIVERSITY OF MICHIGAN LIBRARIES

physiologists as they are produced? What can be done to increase participation of plant physiologists in the rapidly expanding field of agricultural chemical industry? It is hoped that these questions can be dealt with in later reports.

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

Howard S. Reed.—The American Society of Plant Physiologists records with sorrow the passing of one of its members, Howard S. Reed, Emeritus Professor of Plant Physiology of the University of California. Professor Reed died on May 12, 1950 in Berkeley, California at the age of 73.

Botany.—J. BEN HILL, LEE O. OVERHOLTS, HENRY W. POPP. Second Edition. McGraw-Hill Book Company, Inc., 330 West 42nd Street, New York 18, New York. 710 pages, 1950. $5.00.

This book comprises the subject matter of a two-semester college course in general botany. The second edition includes the many advances in botany which have been made in recent years in such fields as photosynthesis, respiration, fermentation and enzymes, metabolism, hormones and vitamins, antibiotics, hydroponics and absorption of inorganic substances, biological effects of radiation, use of "tagged elements," cytology, morphology, fossil plants and plant classification. New sections have been added on viruses, actinomycetes, diatoms and the Charales, as well as unusually important sections on meiosis and on the economic significance of the fungi. Greater emphasis than usual is placed upon the physiology of plants and upon ecology. A number of new figures have been added, and improvements have been made in more than 120 of the original figures.

Introductory Biology.—ANDREW STAUFFER, Editor. D. Van Nostrand Company, Inc., 250 Fourth Avenue, New York 3, New York. 722 pages, 1949.

This book aims to give an understanding of the principles of biology. The authors of the book include: Nelly J. Bosma, E. C. Colin, R. Clark Gilmore, S. W. Howe, W. Henry Leigh, P. Meglitsch, H. C. Nelson, J. F. Schuett, Andrew Stauffer, and J. P. Wessel. The book is divided into ten units which are entitled: (1) Introducing living organisms; (2) How organisms secure food; (3) How organisms transport materials within their bodies; (4) How organisms utilize food and excrete wastes; (5) How organisms co-ordinate their activities; (6) How organisms reproduce; (7) How organisms inherit; (8) How species originate; (9) How organisms live with each other; (10) How man may live healthfully. The book includes an extensive subject index and list of supplementary readings.

Biophysical Research Methods.—Fred M. Uber, Editor. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, New York. 667 pages, 1950. $9.50.

The various authors of this book have each written one chapter which can be read as an independent unit without regard to its position in the volume. This group of specialists includes: Harold F. Blum, David R. Briggs, Howard J. Curtis, Earl W. Flosdorf, John W. Gowen, L. H. Gray, Earle C. Gregg, Jr., James D. Hardy, L. V. Heilbrunn, James Hillier, Max Kleiber, E. G. Pickels, Lawrence R. Prouty, Oscar W. Richards, Fred M. Uber, and Adolf F. Voigt. Each chapter covers the following points: (1) the fundamental principle of one method, its underlying assumptions, and perhaps a simple mathematical outline of the theory involved; (2) the types of problems for which the method offers promise of a unique solution or a helpful approach; (3) the demands made on apparatus, materials and technical skill; (4) the limitations of the method in some detail; and (5) briefly, its outstanding accomplishments to date. The volume is intended to serve as a stimulating but critical guide to the group of scientists who must report to physical methods of research for the solution of biological, medical, and agricultural problems. References for commercial sources of equipment and materials have been included by several authors.

Progress in Biochemistry.—Felix Haurowitz. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, New York. 405 pages, 1950. $7.50.

This book is a compilation of results of biochemical research since 1939. The review begins with a brief chapter on isotopes and on the part they play in biochemistry. The main portion of the book deals with the biochemistry of the organic and inorganic body constituents and with their metabolism. This section is followed by chapters on vitamins, on hormones and on enzymes. In general only human and animal biochemistry is discussed. However, in an addendum to the vitamin chapter, biologically active substances from molds, bacteria and plants are briefly treated.

Advances in Enzymology and Related Subjects of Biochemistry, Volume X. Edited by F. F. Nord. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, New York. 533 pages, 1950. $7.50.

This volume, the latest addition to the well known series, includes the following: Blood clotting and related processes by Tage Astrup; Tryptophanase-tryptophane reaction by F. C. Happold; Phosphatase alcaline by Jean Roche and Nguyen-Van Thoai; Synthesis of disaccharides with bacterial enzymes by W. Z. Hassid and M. Doudoroff; Some aspects of streptomycin and other streptomyces antibiotics by N. G. Brink and Karl Folkers; Probleme des Citronensäurecyklus by C. Martius and F. Lynen; Die Phytochemie des Schwefels by Theodor Bersin; Chemical changes in the harvested tobacco leaf. Chemical and enzymic conversions during fermentation and

UNIVERSITY OF MICHIGAN LIBRARIES

aging by W. G. Frankenburg; and Assimilation of hydrocarbons by microorganisms by C. E. ZeBell. The current volume contains both author and subject indices and also a cumulative index for volumes one to ten inclusive.

Jan Ingenhousz—Plant Physiologist. With a History of the Discovery of Photosynthesis. H. S. REED. Chronica Botanica Company, Waltham, Massachusetts. Volume 11, 111 pages, 1949. $3.00.

This separate makes available a book which has long been out of print, being generally known heretofore only through quotation. It includes a brief biographical sketch showing how the researches of Ingenhousz fitted into the scientific work of the times. Some reflections of Ingenhousz' contemporaries such as Priestley, Scheele, Lavoisier, and Senebier, and the conditions of science in which they worked have also been introduced. Explanatory comments following each section of the original text have been inserted for readers who may wish to trace subsequent developments of Ingenhousz' work. Numerous illustrations, many of them obtained from Netherlands archives and never published before, are a feature of special interest.

Organic Chemistry.—PAUL KARRER. Fourth Edition. Elsevier Publishing Company, Inc., 215 Fourth Avenue, New York 3, New York. 973 pages, 1950. $8.50.

In the fourth English edition of this textbook various chapters have been revised and extended and several new sections have been added. The book is divided into four main parts which are: (1) Aliphatic compounds; (2) Carbocyclic compounds; (3) Heterocyclic compounds; and (4) Organic compounds with heavy hydrogen and heavy oxygen. Sections on the following subjects have now been included: polysiloxanes and other organic silicon compounds, diacyl peroxides and peracids, streptomycin, organic compounds containing isotopes of carbon nitrogen and other elements. The most recent investigations have been mentioned wherever possible. The book also contains an extensive subject index and list of dates of important discoveries.

Physical Methods of Organic Chemistry—Part II. Edited by ARNOLD WEISSBERGER. Second Edition. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, New York. 1023 pages, 1949. $12.50.

This edition has been enlarged by five new chapters, new sections in some chapters, and a substantial expansion of many of the other chapters; some chapters have been entirely rewritten and others have been thoroughly revised. New chapters deal with the determination and evaluation of vapor pressure, measurements made with the ultracentrifuge, and by electrophoresis, temperature measurement and temperature control. Sections present electron microscopy nephelometry; the determination and evaluation of light scattering, and the use of light sources. This volume is de-

voted to a comprehensive presentation of the techniques which are used in the organic laboratory and which are available for the investigation of organic compounds. The authors give the theoretical background for an understanding of the various methods and operations and describe the techniques and tools, their modifications, applicability and limitations of procedures and manner of implementation. An extensive subject index is included in the book.

A.S.P.P. Election 1950–51.—Newly elected officers of the society are: KENNETH V. THIMANN, President; DANIEL I. ARNON, Vice-President; Member of the Executive Committee, DONALD B. ANDERSON, 1953; Member of the Editorial Board, PAUL J. KRAMER, 1953.

UNIVERSITY OF MICHIGAN LIBRARIES

CAN SOCIETY OF PLANT PHYSIOLOGISTS

[illegible] for the advancement of Plant Physiology [illegible] and applied fields of botanical science

---

OFFICERS FOR 1950–51

[illegible] V. THIMANN,
Harvard University, Cambridge, Mass.

[illegible] DANIEL I. ARNON,
University of California, Berkeley, Calif.

[illegible] C. BROYER,
University of California, Berkeley, Calif.

[illegible]-Treasurer: DR. J. FISHER STANFIELD,
Miami University, Oxford, Ohio

[illegible] interested in Plant Physiology is eligible for [illegible]. Candidates for membership must be recommended by [illegible] approved by executive committee, or by the vote of the [illegible] annual or special meeting.

[illegible] of the annual dues to the Society entitles the members to [illegible] journal, PLANT PHYSIOLOGY. They will receive in addition the [illegible] bulletins and announcements sent out periodically by the [illegible]. [illegible] are invited to take a personal interest in the growth and [illegible] of the Society.

[illegible] PLANT PHYSIOLOGY should be addressed to PROF. WALTER [illegible], Department of Botany, State University of Iowa, Iowa City, Iowa. [illegible] and photographs should not exceed 8.5 × 11 inches in size, should be [illegible] correctly on the back, and separate typewritten legends included [illegible] text of the manuscript. Tables and cuts should be used sparingly, [illegible] text presented in concise form. Instructions to authors for preparation of manuscripts are given in the January number, Volume [illegible], 1950, [illegible] Physiology, pages 190–191.

UNIVERSITY OF MICHIGAN LIBRARIES

CONTENTS—(*Continued*)

Nitrogen Level and Season on the Composition
Leaves, University of Connecticut, Storrs ____

Effects on Transpiration and Survival of Seedl
Pine Species. JOHNSON PARKER, Duke Uni
Carolina ____

Carbon Dioxide Evolution and the Absorption of Nitrate
Ions and I. W. WANDER, University of
Massachusetts, and University of Florida
Station, Lake Alfred, Florida ____

Growth-Regulating Substances on the Transpiration
Phaseolus communis. L. MARY ANNE PLATT
Evanston, Illinois ____

Solanum pseudo-capsicum. I. Growth Experiments with
Burr, Cornell University, Ithaca, New York

Chlorotic Orange Leaves and Some Observations
Paraffin Preparation Technique to the Interpre
F. P. GRIFFIN, WALTER REUTHER, and A. W.
Fruit Industry, U. S. Department of Agri-
Florida and Beltsville, Maryland ____

Use of Thermistors in Temperature Investiga-
Reference to a Technique for High Sensitivity
Measurement. R. B. PLATT and J. N. WEIS,
Emory University, Georgia, and University of
Tennessee ____

Apparatus for Measuring Nectar Yield. C. A. SWANSON
Ohio State University, Columbus, Ohio ____

Corn Embryos. R. E. MAXWELL, Iowa Agri-
Station, Ames, Iowa ____

Effects of Certain Nitrogenous Acid on the Invertase, Phosphory-
Peroxidase Activity in the Stems and Leaves of
Green Plants. W. B. NEELY, C. D. BALL, C. L.
and M. SELL, Michigan State College, East Lansing,
Michigan ____

Index ____